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Regulation and critical role of potassium homeostasis in apoptosis

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Abstract

Programmed cell death or apoptosis is broadly responsible for the normal homeostatic removal of cells and has been increasingly implicated in mediating pathological cell loss in many disease states. As the molecular mechanisms of apoptosis have been extensively investigated a critical role for ionic homeostasis in apoptosis has been recently endorsed. In contrast to the ionic mechanism of necrosis that involves Ca^{2+} influx and intracellular Ca^{2+} accumulation, compelling evidence now indicates that excessive K^+ efflux and intracellular K^+ depletion are key early steps in apoptosis. Physiological concentration of intracellular K^+ acts as a repressor of apoptotic effectors. A huge loss of cellular K^+, likely a common event in apoptosis of many cell types, may serve as a disaster signal allowing the execution of the suicide program by activating key events in the apoptotic cascade including caspase cleavage, cytochrome *c* release, and endonuclease activation. The pro-apoptotic disruption of K^+ homeostasis can be mediated by over-activated K^+ channels or ionotropic glutamate receptor channels, and most likely, accompanied by reduced K^+ uptake due to dysfunction of Na^+, K^+-ATPase. Recent studies indicate that, in addition to the K^+ channels in the plasma membrane, mitochondrial K^+ channels and K^+ homeostasis also play important roles in apoptosis. Investigations on the K^+ regulation of apoptosis have provided a more comprehensive understanding of the apoptotic mechanism and may afford novel therapeutic strategies for apoptosis-related diseases.

Contents

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1. Introduction

Programmed cell death or apoptosis normally contributes to tissue development and homeostasis but may also mediate pathological cell loss after chemical, physical, environmental, or genetic insults (Raff et al., 1993; Thompson, 1995). Apoptosis was originally defined by morphological characteristics, including cell body shrinkage, nuclear condensation, chromatin margination, DNA fragmentation (laddering), and formation of membrane-bound cellular remnants (apoptotic bodies) (Kerr et al., 1972; Majno and Joris, 1995). Sub-cellular and molecular regulations of apoptosis such as release of cytochrome *c* from mitochondria, formation of the apoptosome, participation of a number of caspases, and modulation by *bcl-2* family genes have been extensively studied (Jacobson and Evan, 1994; Hengartner et al., 1992; Johnson Jr. et al., 1995; Li and Yuan, 1999; Adams and Cory, 2002). However, until a few years ago little attention had been paid to ionic regulation of apoptosis. Research from our group as well as others' groups has established the novel concept that apoptosis not only is regulated by a number of molecular genes but is also controlled or regulated by cellular ionic homeostasis, particularly the K^+ homeostasis.

Excellent reviews have summarized the critical role of K^+ in pro-apoptotic cellular and biochemical events such as caspase activation and mitochondrial alterations (Bortner and Cidlowski, 1998; Hughes Jr. and Cidlowski, 1999; Montague et al., 1999). The present review is to provide a comprehensive inspection on studies implying possible links between K^+ efflux and apoptosis-related events. The focus of this review is the recent investigations on the mechanism and pathways of pro-apoptotic K^+ efflux and intracellular K^+ depletion. Roles of voltage-gated and other types of K^+ channels, ligand-gated glutamate receptor channels, and the K^+ uptake system of Na^+, K^+-ATPase in apoptosis will be discussed in detail. The potential clinical significance of the K^+ regulation of apoptosis will also be reviewed. It is expected that the increasing attention on the ionic basis of apoptosis, together with investigations on the molecular basis of apoptosis, will lead to a better and more complete understanding of apoptotic pathogenesis and may eventually promote developments of novel therapeutic strategies for various apoptosis-related diseases.

2. Evidence linking K^+ homeostasis to apoptosis

K^+ is the predominant ion inside the cell (~140 mM); in striking contrast, intracellular Na^+, Ca^{2+}, and Cl^- concentrations are typically one to several orders of magnitude lower than K^+ (Fig. 1). Therefore, intracellular K^+ ($[K^+]_i$)

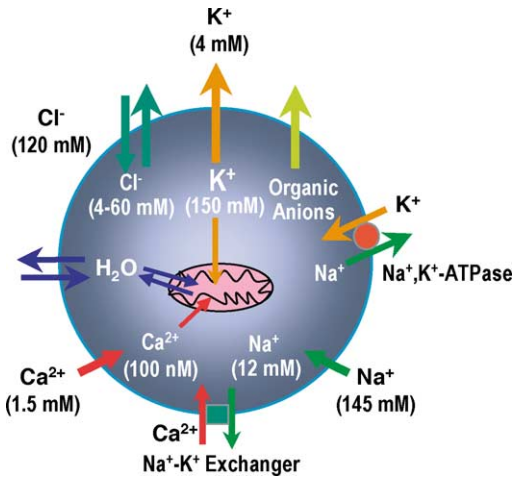

Fig. 1. Ionic distributions across the membranes and pathways for K^+ fluxes. Driven by their concentration gradients as well as electrical forces, K^+, Cl^-, and organic anions flow from the cytoplasm to extracellular space via ion channels and exchangers. Active ionic movements can be achieved by corresponding transporters/pumps. K^+ may also move into mitochondria upon activation of mitochondrial K^+ channels such as the mK_{ATP} channel. Water moves following the osmolarity changes in the cytoplasm and mitochondria. The K^+, Cl^-, and water efflux are most likely responsible for apoptotic cell volume decrease. Because of excessive K^+ and Cl^- efflux over the water movement, there are significant decreases in intracellular concentrations of K^+ and Cl^- in apoptotic cells. Moving of K^+ and water into the mitochondrial matrix and intramitochondrial space may cause mitochondrial swelling, lost of mitochondrial potential, disruption of the outer membrane, and probably release of some apoptotic factors such as cytochrome *c* into the cytoplasm (see text for detail). Activation of Ca^{2+} and Na^+ channels in the plasma membrane results in influx of Ca^{2+} and Na^+, which is believed a trigger for necrosis. Ca^{2+} influx into the mitochondrial may also cause damage there related to apoptosis. The homeostasis of K^+, Ca^{2+}, and Na^+ is maintained by balance between efflux and influx across the membranes. The energy-dependent Na^+, K^+-ATPase may play a critical role in K^+ and Na^+ homeostasis, and, consequently, affect Ca^{2+} homeostasis. The cytoskeleton and other membrane channels/transporters may contribute to the cell volume control and various apoptotic events, which are not the topics of this review and are not illustrated in the figure.

and associated water movement are the major determinants of cytoplasm volume. The extracellular environment contains low levels of K^+ and high levels of Na^+; the net electrochemical gradient across the membrane is favorable for moving K^+ out of the intracellular space. The K^+ and Na^+ gradients across the membrane are mainly maintained by continuous activities of the Na^+, K^+-ATPase (Robinson and Flashner, 1979). Accordingly, an excessive K^+ efflux and/or dysfunction of Na^+, K^+-ATPase will lead to depletion of $[K^+]_i$ (Fig. 1). The K^+ loss-generated osmolarity alterations are favorable for moving water out from the cell and believed a major contributor to physiological as well as pathological cell volume decrease; meanwhile, Cl^- movement may also significantly affect cell volume regulation (Yu and Choi, 2000; Okada and Maeno, 2001).

2.1. K^+ homeostasis and cell proliferation

A possible role for K^+ in apoptosis may be derived from its involvement in cellular proliferation. The balance between proliferation and cell death controls the number of cells. These two processes, although leading to opposite outcomes, share a number of "Janus genes" such as *p53* and c-*myc* (Ferrari and Greene, 1994; Kroemer et al., 1995). From a morphological point of view, apoptosis and mitosis have a number of similar characteristics such as cytoskeletal changes, rounding up of the cell, nuclear envelope breakdown, and chromatin condensation. Depending on the conditions, c-*myc* can activate either cell proliferation or apoptosis (Evan et al., 1994), and *p53* either arrests the cell cycle or activates apoptosis (Yonish-Rouach et al., 1993). These similarities support the speculation that apoptosis, or at least some forms of apoptosis, might represent an aberrant cell cycle, involving out-of-phase expression of mitotic entities in post-mitotic cells (such as neurons) with lethal consequence. Thus apoptosis may be regarded as an abortive or premature mitosis or "mitotic catastrophe" (Ucker, 1991; Kroemer et al., 1995).

The concept of abortive mitosis is important and relevant to the K^+ regulation of apoptosis because K^+ channel activity plays vital regulatory roles in cell proliferation (Chiu and Wilson, 1989; Nilius and Wohlrab, 1992; Wang et al., 1997). DeCoursey et al. (1984) and others (Nilius and Wohlrab, 1992; Ribera and Spitzer, 1992; Pappone and Ortiz-Miranda, 1993) have shown, mainly in the cells of immune system, that outward delayed rectifier or other K^+ channels regulate mitogenesis and differentiation. Blocking K^+ channels or lack of functional K^+ channels usually prevents cells from proliferation. A decrease in intracellular K^+ might be one of the key events in initiating mitosis in mature central neurons (Cone Jr. and Cone, 1976), and elevated extracellular K^+ inhibits cell proliferation (McCabe and Young, 1994). In low serum media, which stimulated cell differentiation, the delayed rectifier K^+ (I_K) channel was expressed or markedly up-regulated (Smith-Maxwell et al., 1991; Quidt, 1994). On the other hand, lack of serum in the culture medium is also an insult for apoptosis of cortical neurons (Koh et al., 1995), mediated by increased I_K currents (Yu et al., 1997 and see Section 2.2).

2.2. K^+ efflux and cellular K^+ depletion induce cell shrinkage and apoptosis

Early studies, using toxins and selective K^+ ionophores, linked K^+ efflux to cell shrinkage and other apoptotic events. *Staphylococcus aureus* α-toxin evoked protracted, irreversible ATP depletion in both activated and resting human T lymphocytes (Jonas et al., 1994). The plasma membrane of toxin-damaged cells became permeable for monovalent ions but not for Ca^{2+} and propidium iodide. The permeabilization event was followed by internucleosomal DNA degradation characteristic of programmed cell death. The data indicated that formation of very small α-toxin pores might trigger programmed cell death in lymphocytes (Jonas et al., 1994). Beauvais et al. (1995) tested the hypothesis in human eosinophils that apoptotic cell shrinkage was linked to osmotic changes due to leakage of internal ions. They observed that cytokine deprivation-induced apoptotic shrinkage was inhibited in a dose-dependent manner by several K^+ channel blockers (4-aminopyridin or 4-AP, sparteine, and quinidine). To induce apoptosis in murine tumor cells, Duke et al. (1994) found that these cells could tolerate increases in intracellular free Ca^{2+} ($[Ca^{2+}]_i$) approaching 1 mM induced by the Ca^{2+} ionophore A23187, but they died from apoptosis induced by valinomycin. Valinomycin is an antibiotic K^+ ionophore produced by *Streptomyces*. It is a cyclic polypeptide-like molecule whose folded conformation forms an inner cavity that can accommodate K^+ but not other ions. Up to now, valinomycin-induced apoptotic changes have been reported in a variety of cell types (Table 1). Other K^+ ionophores such as amphotericin-B also enhance K^+ efflux and induce apoptosis (Marklund et al., 2001a).

A number of studies have explored the relative importance of intracellular Ca^{2+}, K^+, and other ions in apoptosis. The K^+ ionophore beauvericin induced apoptosis in cholangiocytes that was accompanied by decreased $[K^+]_i$ and increased $[Ca^{2+}]_i$. Because high K^+ medium (120 mM), which was toxic, did not reduce cell death but removal of extracellular Ca^{2+} did, the authors suggested that $[Ca^{2+}]_i$ accumulation was responsible for the death (Que et al., 1997). They also noted, that although Ca^{2+} was important, changes in Ca^{2+} alone were not sufficient to cause apoptosis. Barbiero et al. (1995) monitored changes in intracellular Ca^{2+}, H^+, Na^+, and K^+ in L cells undergoing cell cycle arrest and apoptosis. They found that during the apoptotic process lasting up to 4 days, there was a transient increase in $[Ca^{2+}]_i$ from 97 to 150 nM in the initial 6 h, a transient decrease in pH by 0.2–0.4 units at 24 h, and a persistent (up to 3 days) increase in intracellular Na^+ ($[Na^+]_i$) from 15 to 30 mM. There was a marked reduction in $[K^+]_i$ from 110 mM to less than 50 mM in a subpopulation of

Table 1
K^+ efflux-mediated apoptosis in different cell types

Apoptotic insult	Cell type	Observation	References
K^+ ionophores: valinomycin, nigericin, etc.	Mouse peritoneal macrophages; rodent lymphocytes/thymocytes and tumor cell lines; hepatoma cells; pre-B BAF3 cells; AH-130 cells; central neurons; human pulmonary mesothelioma cell line	K^+ efflux led to cell volume decrease, IL-1β maturation, caspase and endonuclease activation, DNA fragmentation, mitochondrial damage, and apoptosis	Allbritton et al., 1988; Ojcius et al., 1991; Deckers et al., 1993; Duke et al., 1994; Perregaux and Gabel, 1994; Inai et al., 1997; Yu et al., 1997; Furlong et al., 1998; Dallaporta et al., 1999; Marklund et al., 2001a,b
Staphylococcal α toxin	T lymphocytes	The staphylococcal α toxin-formed pores allowed passage of K^+ and Na^+ and induced DNA degradation	Jonas et al., 1994
Cytokine withdrawal	Human eosinophils	Marked cell shrinkage due to leakage of internal ions; K^+ channel blockers inhibited the shrinkage	Beauvais et al., 1995
Thymidine or VP-16	L cells	Intracellular K^+ dropped to $\leq$50 mM in a subpopulation of cells	Barbiero et al., 1995
Lipopolysaccharide (LPS)	Human monocytes	High K^+ medium prevented IL-1β maturation induced by LPS	Walev et al., 1995
Dexamethasone	CEM-C7A lymphoblastoid cells	Loss of intracellular K^+ in apoptotic cells	Benson et al., 1996
Serum deprivation, β-amyloid, NMDA, AMPA, kainate, and glutamate	Rodent cortical neurons	Apoptotic insults stimulated K^+ efflux and reduced $[K^+]_i$ via over-activated K^+ channels or ionotropic glutamate receptors, leading to caspase activation and apoptosis	Yu et al., 1997, 1998, 1999b
Staurosporine	Central neurons; vascular smooth muscle cells; mouse embryos	Enhanced outward K^+ current, increased K^+ efflux, caspase activation, DNA laddering, and apoptosis	Yu et al., 1997; Ekhterae et al., 2001; Trimarchi et al., 2002
UV irradiation	HL-60 cells; human monoblastoid cell line (U937)	Decreases in cellular Na^+ and K^+ in apoptotic HL-60 cells; roles of Na^+, K^+-ATPase and Ca^{2+}-dependent K^+ channels; low cellular K^+ and Cl^- content and high Na^+ in apoptotic U937 cells	McCarthy and Cotter, 1997; Fernandez-Segura et al., 1999
Glucocorticoids, topoisomerase inhibition, staurosporine; Fas-crosslinking	Lymphocytes/thymocytes, Jurkat cells, and T cell hybridoma cells	Intracellular K^+ depletion (down to 35 mM); caspase/nuclease activation, and disrupted mitochondrial potential in shrunken cells, induction of apoptosis	Hughes Jr. et al., 1997; Bortner et al., 1997; Dallaporta et al., 1998, 1999; Hughes Jr. and Cidlowski, 1999; Mann et al., 2001
Sorbitol	HeLa cells	Pro- and anti-apoptotic effect of K^+	Bilney and Murray, 1998
Ceramide	Central neurons; thymocytes	Enhanced outward K^+ current, activation of capsase-3, DNA laddering, mitochondrial damage and apoptosis	Yu et al., 1999b; Dallaporta et al., 1999
Anti-Fas/CD95	Jurkat T cells	Cell shrinkage and K^+ depletion, inhibited K^+ uptake due to block of Na^+, K^+-ATPase; effect of PKC on cell death through inhibition of cell shrinkage and K^+ efflux	Bortner and Cidlowski, 1999; Gomez-Angelats et al., 2000; Bortner et al., 2001
A23187	Jurkat T cells	Cell shrinkage, K^+ efflux, mitochondrial damage, DNA damage, and apoptosis	Bortner and Cidlowski, 1999
Thapsigargin	Jurkat T cells	Cell shrinkage, K^+ efflux, mitochondrial damage, DNA damage, and apoptosis	Bortner and Cidlowski, 1999
TNF	Human and rodent T cell; hybridoma-derived PC60 R55/R75 cells	Nigericin, valinomycin or ouabain enhanced TNF-induced apoptosis	Penning et al., 2000
Doxorubicin or trophic hormone deprivation	Oocytes and granulose cells	Significant loss of cellular K^+ during apoptosis; high K^+ medium effects; K^+-dependent and independent events	Perez et al., 2000

Table 1 (*Continued*)

Apoptotic insult	Cell type	Observation	References
Chemotherapeutic anti-cancer drugs (cisplatin, carboplatin)	Human pulmonary mesothelioma cells (P31)	Stimulating K^+ efflux potentiated caspase activation and apoptosis	Marklund et al., 2001b
Etoposide	Jurkat T cells	Cell shrinkage, K^+ efflux, phosphatidylserine externalization, mitochondrial depolarization, and apoptosis	Thompson et al., 2001
Ouabain	Central neurons, thymocytes	Cell shrinkage, K^+ depletion, mitochondrial damage, caspase activation, DNA damage, apoptosis or hybrid death	Mann et al., 2001; Xiao et al., 2002c
Antifungal, antibiotic amphotericin B	Mesothelioma cells (P31)	K^+ efflux, K^+ depletion and apoptosis	Marklund et al., 2001a
Hydrogen peroxide, diamide	Mouse embryos	A pronounced transient K^+ efflux through TEA-sensitive K^+ channels accompanied by apoptotic shrinkage; increases in extracellular K^+	Trimarchi et al., 2002

This table lists representative published studies on the relationship between K^+ and apoptosis in a relatively chronological order.

cells in 3 days. The authors proposed that K^+ extrusion possibly played a role in the final loss of cell volume. In the human monoblastoid cell line U937, ultraviolet (UV) light-irradiated apoptotic cells showed low intracellular K^+ (50% reduction), Cl^- (27% reduction), and high intracellular Na^+ (a four-fold increase), but no significant change in $[Ca^{2+}]_i$ as measured by electron probe X-ray microanalysis (Fernandez-Segura et al., 1999). In a separate study on the role of the three cations, Ca^{2+}, Mg^{2+}, and K^+, K^+ deprivation was identified as the most detrimental to cellular growth and viability. Consistent with this conclusion, *bcl-2* over-expression showed less protection in a K^+-free medium (Ishaque and Al-Rubeai, 1999), which facilitated K^+ efflux and intracellular K^+ loss.

Intracellular Ca^{2+} plays important roles in apoptotic events such as mitochondria damage and activation of caspase and nuclease. The involvement of Ca^{2+} in apoptosis, however, has been conflicting and confusing (Yu et al., 2001), which is not the focus of this review. As for the intracellular Na^+ concentration, in addition to the increases reported in above studies, decreases in $[Na^+]_i$ were also observed in apoptotic lymphoma cells (Bortner et al., 1997) and Jurkat cells (Gomez-Angelats et al., 2000). Apoptotic cells are likely to lose intracellular Cl^- as well that may contribute to apoptotic cell volume decrease and some other events (Yu and Choi, 2000; Okada and Maeno, 2001; and see Section 8). Limited information is available for the influence of other ions on apoptosis (Yu et al., 2001).

2.3. Decreased intracellular K^+ concentration in apoptotic cells

Due to the lack of selective K^+ imaging dyes for accurate assays of intracellular K^+ concentration, most studies have measured intracellular K^+ content as a reflection of concentration. Since the cell membrane behaves like an osmometer and water will move out following K^+ efflux, it can be questioned whether a decrease in cellular K^+ concentration occurs during the apoptotic process. Available evidence now supports the idea that a drastic reduction in $[K^+]_i$ does take place in apoptotic cells. Hughes Jr. et al. (1997) estimated a 95% loss in K^+ content associated with only a 33% loss of cell volume, arguing favorably for a decrease in $[K^+]_i$. The low K^+ concentration in apoptotic cells agrees with an early study that showed intracellular K^+ level was reduced to 50 mM during apoptosis in a fibroblast cell line (Barbiero et al., 1995). In further studies, Hughes Jr. and Cidlowski (1999) estimated that intracellular K^+ could be as low as 35 mM in shrunken apoptotic thymocytes. Cell volume loss in dexamethasone-treated apoptotic CEM-C7A lymphoblastoid cells was associated with a net loss of cellular K^+. The mean intracellular K^+ content of the cells significantly decreased (~15% by 24 h, ~40% by 48 h), the cellular water volume was unchanged at 24 h but slightly less at 34 h (~20% decrease) (Benson et al., 1996), suggesting a reduction in intracellular K^+ concentration. Supporting a low K^+ concentration in apoptotic cells, Montague et al. (1999) reported that shrunken cells lost K^+ and became hypotonic, consistent with an excess of K^+ efflux over water efflux and a reduction in $[K^+]_i$. In thymocytes and T hybridoma cells, several apoptotic insults (glucocorticoids, topoisomerase inhibition, and Fas-crosslinking) all induced cellular K^+ loss as measured by the K^+ dye PBFI (Dallaporta et al., 1998). After the earliest changes in mitochondrial membrane potential, the loss of cytosolic K^+ occurred before cell shrinkage, consistent with a role for K^+ efflux in cell volume reduction. K^+ loss was also seen before the production of reactive oxygen species (ROS). In these apoptotic models, the K^+ loss exhibited a strong correlation with nuclear DNA fragmentation (Dallaporta et al., 1998) (Fig. 2).

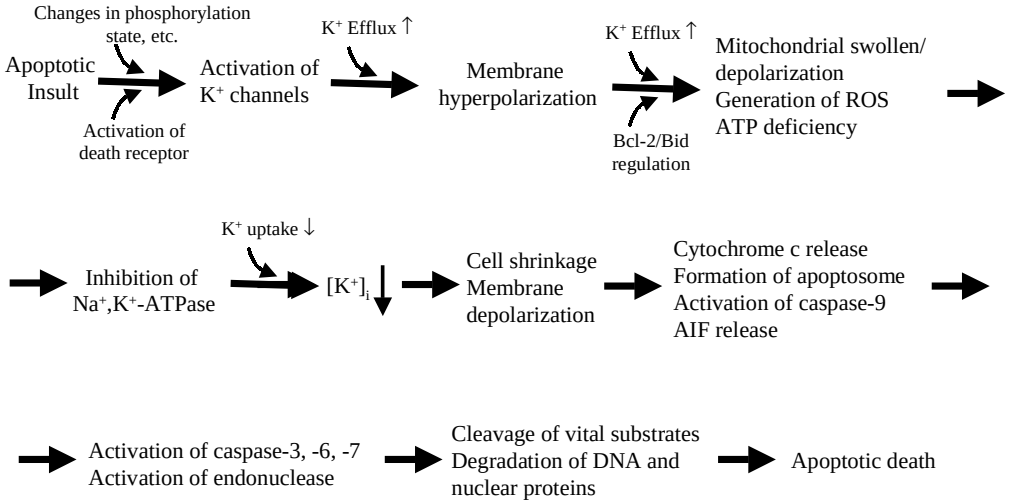

Fig. 2. Chronological events associated with the K^+ regulation of apoptosis. A number of apoptotic insults stimulate activation of a variety of K^+ channels and/or ionotropic glutamate receptors, likely mediated by signal transduction systems including those that change phosphorylation states of the targeted channels. Activation of "death receptors" such as TNFα and Fas receptors may also initiate the K^+ mechanism for apoptosis. The higher K^+ conductance initially drags the membrane potential towards negative direction of the K^+ equilibrium potential. The mitochondrial permeability transition including inflow of K^+ and water into the mitochondria cause mitochondrial swelling and depolarization. This apoptotic process may be counter-regulated by *bcl-2* and *Bid*. The insult and damage to mitochondria lead to ROS generation and deficient energy production, which consequently block the Na^+, K^+-ATPase. Most likely, the dysfunction of Na^+, K^+-ATPase delivers a blow to the already deteriorating situation of excessive K^+ efflux, resulting in depletion of intracellular K^+, cell volume decrease, and cell membrane depolarization. In the presence of certain minimum level of dATP/ATP, mitochondrial released cytochrome *c* and Apaf-1 form apoptosome and cleave procaspase-9. Release of apoptosis-inducing factor (AIF) causes caspase-independent apoptosis that may also be regulated by K^+ homeostasis. In reduced intracellular K^+ concentration (∼50 mM), activation of execution caspases such as caspase-3 and nucleases takes place. DNA damage and nuclear collapse eventually lead to apoptotic death. Information about potential contributions from other ions such as Ca^{2+}, Na^+, Mg^{2+}, and Cl^- can be found in the text and listed reviews, and are not shown in the diagram.

2.4. Intracellular K^+ depletion and activation of key apoptotic enzymes

A puzzling question that has arisen since the emergence of a possible relationship between K^+ and apoptosis is how might one link a bulky intracellular cation such as K^+ to the delicately and tightly regulated cell death program? Because of the ample amount of this intracellular cation, it seemed that a loss of K^+ should not be a serious concern for the survival of a cell.

The answer may ironically be found in the control of suicide activity that is committed by a well-orchestrated inherited program. It is imaginable that such a suicide program should be designed with high margins of safety and not to be turned on easily. Potassium, as the most abundant cation inside the cell, is in a unique position to safely but forcefully deliver this vital signal to the cell. Because of its abundance, cells can tolerate loss of tens of millimolar concentrations of intracellular K^+ for up to 20–30% of total cellular K^+ without deleterious consequences and continue to live. This threshold satisfies the necessary safety margin in initiating the suicide mechanism. On the other hand, when cells lose unmanageable amounts of intracellular K^+, i.e. $\sim\geq$50% of total cellular K^+, the struggling cells have to initiate and execute the K^+-sensitive suicide cascade as a way of cell elimination.

The clues for the missing link between a massive K^+ loss and induction of apoptosis were scattered in early studies that showed that in several cell types reagents promoting cellular K^+ loss stimulated maturation and release of the inflammatory factor interleukin 1β (IL-1β) (Perregaux and Gabel, 1994; Walev et al., 1995). IL-1β, later named caspase-1, is a member of the signaling family for the induction of apoptosis (Jacobson and Evan, 1994). In human peripheral blood mononuclear cells, the IL-1β activation was not affected by Na^+ or Ca^{2+} influx; it was, however, stimulated by agents that reduce $[K^+]_i$ such as valinomycin and nigericin (Walev et al., 1995). IL-1β activation was blocked when cells were suspended in a high K^+ medium or exposed to the K^+ channel blocker, tetraethylammonium (TEA) or 4-AP. It was suggested that a net decrease in $[K^+]_i$ was necessary to activate the post-translational maturation of IL-1β (Walev et al., 1995). This is in line with earlier studies that K^+, like Ca^{2+}, is a regulator of a number of enzymes in different cell types (Lubin, 1967; Ledbetter and Lubin, 1977; Albano et al., 1977; Duggan, 1977), and ions can influence protein structure and profoundly alter the activity of proteases and nucleases (Hughes Jr. et al., 1997). A recent study suggested that the K^+-stimulated IL-1β maturation was mediated by a Ca^{2+}-independent phospholipase A2 (Walev et al., 2000).

Works from Cidlowski's group have demonstrated that K^+ inhibited the in vitro activation of pro-caspase-3-like enzymes in a dose-dependent manner ($K_i \cong 40$ mM) and that caspase and nuclease activations were totally inhibited at physiological intracellular K^+ concentrations (e.g. 140 mM) (Hughes Jr. et al., 1997). Several apoptotic insults, dexamethasone, thapsigargin, and staurosporine, induced shrinkage of lymphocytes due to significant reduction of $[K^+]_i$, and apoptosis was inhibited when K^+ efflux was blocked (Bortner et al., 1997). DNA fragmentation was detected after $[K^+]_i$ was reduced from the normal level of ~140–56 mM, which was suppressed by KCl with a K_i of ~70 mM (Hughes Jr. et al., 1997).

Conversely, caspases such as IL-1β may modulate ion channel activities. Both the A-type and delayed rectifier K^+ currents were increased by IL-1β in a voltage- and dose-dependent manner, presumably via a direct mechanism (Szucs et al., 1992). Possible effects of caspase family members on ion channel function and ion homeostasis are not well understood and merit further investigation.

2.5. Is K^+ depletion a trigger or consequence of apoptosis?

A fundamental issue has been whether cellular K^+ depletion acts as a trigger for apoptosis or merely a consequence of the process. The induction of apoptosis in many cell types by valinomycin alone (Table 1) suggests that disruption of K^+ homeostasis itself is sufficient for causing apoptosis. Supporting this proposition, overexpression of a K^+ channel in the cell plasma membrane may induce or promote apoptosis (Yu and Choi, 1999; Nadeau et al., 2000; Pal et al., 2002). Other studies, however, suggest that additional factors are needed for activation of caspases and internucleosomal cleavage enzymes. Incubation of pro-caspase-3-like enzymes in a low K^+ buffer was ineffective in activating the enzyme; similarly, placing inactive nuclease in a K^+-deficient buffer was not sufficient in triggering its DNA degrading properties (Hughes Jr. et al., 1997). Cells placed in a hypotonic solution lose significant K^+ but are not necessarily undergoing apoptosis (Bortner et al., 1997). In a cell free system of apoptosis, physiological concentrations of K^+ (100–140 mM) inhibited chromatin condensation and DNA fragmentation of HeLa nuclei exposed to mitochondrial supernatants treated with atractyloside that induced mitochondrial permeability transition and cytochrome *c* release. The apoptosis-inhibitory effect of KCl was dose-dependent, with an ED_{50} of ~80 mM. Lowering K^+ concentration itself was not sufficient to activate endonucleases; however, it facilitated their activation by mitochondrial supernatants (Dallaporta et al., 1998). Thus, it seems although cellular K^+ depletion is necessary and probably universal in apoptosis, in many cases separate apoptotic activating signals may be needed and act together with low K^+ to initiate the death program.

3. Voltage-gated K^+ channels and the pro-apoptotic K^+ efflux

3.1. Voltage-gated K^+ channels provide a pathway for pro-apoptotic K^+ efflux

To understand the K^+ mechanism underlying apoptosis, it is necessary to delineate the machinery that carries out the K^+ outflow. To accomplish the cellular K^+ loss, the involved channels must be able to allow massive K^+ efflux. A good candidate of such channels is the voltage-gated outward delayed rectifier or I_K channel. The I_K channel belongs to the voltage-gated K^+ (Kv) channels and is a big family consisting of *Shaker*, *Shab*, and *Shaw* channels widely expressed in many cells (Jan and Jan, 1997). I_K channels are opened in a voltage-dependent manner and conduct the most K^+ efflux due to their high K^+ conductance and non- or slow-inactivating behavior during membrane depolarization. Our group reported the first evidence revealing a critical role of I_K channels in the induction of neuronal apoptosis. We demonstrated that serum deprivation- or staurosporine-induced apoptosis of cortical neurons (death in 24–48 h) was associated with an early enhancement (during 3–12 h exposures) of I_K current and loss of cellular K^+ (Yu et al., 1997). Attenuating the outward K^+ current with TEA or elevated extracellular K^+ reduced apoptosis, even if associated increases in $[Ca^{2+}]_i$ were prevented (Yu et al., 1997). A similar I_K enhancement was verified with other apoptotic insults such as the apoptotic messenger ceramide and β-amyloid peptides (Yu et al., 1998, 1999b).

Subsequent studies support the role for Kv channels in apoptosis (Table 2). For example, enhancement of outward K^+ currents may mediate UV light irradiation-induced apoptosis in myeloblastic leukemia cells (Wang et al., 1999). Presenilin-1 or -2 increased outward K^+ current densities in HEK-293 cells, suggesting a possible link between presenilin, K^+ channel, and cell death in Alzheimer's disease (Malin et al., 1998). The Alzheimer's disease-associated β-amyloid fragments also enhanced K^+ conductance in neurons and astrocytes (Furukawa et al., 1996; Colom et al., 1998; Jalonen et al., 1997; Yu et al., 1998; but see Ramsden et al., 2001 for the K^+ current enhancement linked to physiological functions). Infectious bursal disease virus (IBDV), a member of the family *Birnaviridae*, induced apoptosis in chicken peripheral lymphocytes and embryo fibroblasts. IBDV incubation of several hours eliminated the inactivation of an outward delayed rectifier K^+ current and accelerated the opening phase of the current (Repp et al., 1998). The changes lasted for many hours, allowing a significant K^+ efflux. TNFα-induced apoptosis in rat liver cell line HTC cells was accompanied by two- and five-fold increases in K^+ and Cl^- currents, respectively (Nietsch et al., 2000). It was suggested that activation of K^+ and Cl^- channels is an early response to TNF signaling, participating in pathways leading to TNF-mediated cell death. Consistently, stimulating K^+ efflux by nigericin,

Table 2
K^+ channels and K^+ permeable receptor channels indicated in apoptosis

Subtype of K^+ channel	Cell type	Observation	References
Outward delayed rectifier (I_K) or I_K-like channels	Central neurons; myeloblastic leukemia cells	Significant increases of K^+ currents during early stage of apoptosis	Yu et al., 1997, 1998, 1999b; Wang et al., 1999; McLaughlin et al., 2001
Kv1.1, Kv1.5, Kv2.1	Pulmonary artery smooth muscle cells	Regulation by *bcl-2*	Ekhterae et al., 2001
Kv1.3	Porcine granulosa cells; T-leukemic cells	Block of apoptosis by selective channel blockers	Manikkam et al., 2002; Bock et al., 2002
Kv2.1	Cortical neurons; CHO cell	Expression of functional channels increased vulnerability to apoptosis	Yu and Choi, 1999; Pal et al., 2002
Kv3.3	Cerebellar Purkinje cells	Expression of the Kv3.3b K^+ channel marked the terminal phase of Purkinje cell differentiation and was evident in cells prior to death	Norman et al., 1995
Inward rectifier ROMK1 (Kir1.1)	Hippocampal neurons	Expression of the channel induced apoptosis	Nadeau et al., 2000
Ca^{2+}-activated maxi-K channel	Human and rat vascular smooth muscle cells	Carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP)-induced channel activation and apoptosis; antagonism by TEA and iberiotoxin	Krick et al., 2001
Ca^{2+}-activated IKCa1 channel	T lymphocyte	Blocking the IKCa1 channel prevented cell shrinkage, phosphatidylserine translocation and cell death	Elliott and Higgins, 2003
ATP-sensitive K^+ (K_{ATP}) channel	Cardiac myocytes and COS-1 cells	Irradiation increased K_{ATP} channel activity resulted from increased maximal open probability and decreased ATP inhibition	Fan and Neff, 2000
HERG K^+ channel	Tumor cells	HERG channel conductance markedly promoted H_2O_2-induced apoptosis	Wang et al., 2002
NMDA receptor channels	Cortical neurons	NMDA receptor-mediated K^+ efflux, cellular K^+ depletion, and apoptosis	Yu et al., 1999a
AMAP/kainite receptor channels	Cortical neurons	AMPA/kainate receptor-mediated K^+ efflux, cellular K^+ depletion, caspase activation, and apoptosis	Xiao et al., 2001

This list is based on published data; it is likely that more channels and transporters or subtypes of channels and their regulatory mechanisms will be identified in future investigation.

valinomycin, or ouabain enhanced TNF-induced apoptosis in human and rodent tumor cell lines (Penning et al., 2000). Conversely, TNFα was protective against the secondary death of retinal ganglion cells after axotomy of the optic nerve in vivo as well as in vitro in a mixed retinal cultures model. In accordance with the protection, TNFα showed inhibitory action on the outward delayed rectifier-like K^+ currents, while a K^+ channel opener abolished the neuroprotection (Diem et al., 2001). TNFα also increased the A-type transient outward K^+ current (I_A) in cortical neurons and reduced *N*-methyl-D-aspartate (NMDA) toxicity (Houzen et al., 1997). This change in the I_A channel may not affect K^+ homeostasis but presumably causes membrane hyperpolarization responsible for the reduced excitotoxicity.

Additional compelling evidence linking Kv channels to apoptosis came from a study in which the anti-apoptotic gene *bcl-2* was overexpressed in pulmonary artery smooth muscle cells (Ekhterae et al., 2001). The *bcl-2* overexpression attenuated staurosporine-induced K^+ current increase as well as apoptosis. *bcl-2* decreased the amplitude and current density of delayed rectifier K^+ currents, accelerated their inactivation, and downregulated the mRNA expression of the pore-forming α-subunits of the I_K family members Kv1.1, Kv1.5 and Kv2.1. (Ekhterae et al., 2001).

Transfection and modification of K^+ channel genes in cells provide a way of selectively verifying the involvement of a particular channel in apoptosis. Expression of the Kv2.1 channel in HEK293 cells increased the vulnerability of these cells to the apoptotic insult C_2-ceramide (Yu and Choi, 1999); Kv2.1 gene transfection in Chinese hamster ovary cells enhanced vulnerability to apoptosis induced by an NO donor 3-morpholinosydnonimine (Sin-1) or oxidative stress (Pal et al., 2002). In the latter study, the Kv channels involved in the apoptotic process seemed highly subtype

specific; mutated A-type channel without fast inactivation, which should make the channel behave like a delayed rectifier, showed no pro-apoptotic property. The reason for the subtype specificity is unclear and the impact of the K^+ channel modulation on $[K^+]_i$ was not verified. T-leukemic cells genetically deficient for the K^+ channel Kv1.3 were resistant to DNA fragmentation, cytochrome *c* release, mitochondrial membrane potential change, and apoptotic death initiated by the cytostatic drug actinomycin D, while transfection of Kv1.3 restored the sensitivity of these cells to actinomycin D (Bock et al., 2002). In cerebellar Purkinje cells, lurcher gene-induced apoptosis occurred after expression of the Kv3.3 channel, implying a pro-apoptotic effect for these K^+ channels (Norman et al., 1995). Overexpression of the inward rectifier K^+ channel ROMK1 (Kir 1.1) in rat hippocampal neurons induced apoptosis in 48–72 h (Nadeau et al., 2000). Elevating extracellular K^+ from 5.4 to 15.4 mM blocked the apoptotic neuronal death. Interestingly, manipulations of intracellular Ca^{2+} could not affect the cell death (Nadeau et al., 2000).

The K^+ channels capable of mediating the pro-apoptotic K^+ efflux are not limited to the delayed rectifier I_K channels and, in some cases, the A-type I_A channels (Table 2). The large-conductance, voltage- and Ca^{2+}-sensitive K^+ (maxi-K) channels, may mediate the pro-apoptotic K^+ efflux in vascular smooth muscle cells. FCCP, which dissipates the H^+ gradient across the inner membrane of mitochondria, increased the maxi-K currents in these cells and induced apoptosis; both of which were blocked by the K^+ channel blockers TEA and iberiotoxin (Krick et al., 2001). This mechanism may also mediate the apoptosis induced by nitric oxide in these cells (Krick et al., 2002). A recent study showed in T lymphocytes that the Ca^{2+}-activated K^+ channel IKCa1 mediated the apoptotic shrinkage and cell death induced by the Ca^{2+} ionophore calcimycin. Blocking the IKCa1 channel completely prevented cell shrinkage, phosphatidylserine translocation and cell death (Elliott and Higgins, 2003). Interestingly, the apoptosis tested in this study was caspase-independent, suggesting that the K^+ mechanism might regulate different forms of apoptosis.

Two-pore domain K^+ channels may underlie the K^+ efflux related to apoptotic shrinkage in mouse embryos (Trimarchi et al., 2002). The apoptotic message ceramide inhibited inwardly rectifying K^+ currents in cultured oligodendrocytes; ceramide reduced K^+ influx at negative membrane potentials (−50 mV or more negative) without affecting K^+ efflux at positive potentials (Hida et al., 1998), an effect that may cause a pro-apoptotic decrease in $[K^+]_i$.

Conflicting data has been reported indicating that apoptotic insults may inhibit K^+ currents instead of enhancing them. Synthetic N-terminus peptides of Reaper and Grim pro-apoptotic proteins induced or accelerated inactivation of K^+ currents, and it was suggested that Reaper and Grim might initiate apoptosis by blocking K^+ channels (Avdonin et al., 1998). Whether the changes in the channel inactivating kinetics affected $[K^+]_i$ and directly related to apoptosis was unclear. Reaper mutations, like F5A and Y6A examined in this study, had reduced killing ability while retaining the ability to largely inactivate these K^+ currents (Avdonin et al., 1998).

3.2. Phosphorylation state and pro-apoptotic modulation of K^+ channels

As an important mechanism in cell survival and apoptosis, protein phosphorylation may play a principal role in the pro-apoptotic regulation of K^+ channels and K^+ efflux. In cortical neurons, we have shown that tyrosine phosphorylation mediated the modulation of I_K channels during apoptosis; both the I_K current enhancement and apoptotic death were attenuated by the tyrosine kinase inhibitor herbimycin A or lavendustin A (Yu et al., 1999b). In neuronal apoptosis induced by 2,2′-dithiodipyridine (DTDP), a compound inducing intracellular zinc release, an early and robust increase in TEA-sensitive K^+ currents was likely mediated by p38 phosphorylation (McLaughlin et al., 2001). Interestingly, Holmes et al. (1997) observed that expression of voltage-gated K^+ channels in HEK 293 cells decreased cellular protein tyrosine phosphorylation; valinomycin induced a similar effect. Considering that tyrosine phosphorylation of certain cellular proteins is critical for cell survival (Paulson and Bernstein, 1995; Fischer, 1999), this result may imply that the reduced tyrosine phosphorylation may be a mechanism mediating the pro-apoptotic effect of enhanced K^+ efflux and cellular $[K^+]_i$ depletion. We have also shown recently that tyrosine phosphorylation regulates the activity of Na^+, K^+-ATPase in cortical neurons; an inhibition of tyrosine phosphorylation of the Na^+ pump protein substantially suppresses the pump activity, reduces $[K^+]_i$, and leads to neuronal apoptosis (Wang and Yu, 2002; and see Section 6 for the relationship between Na^+, K^+-ATPase and apoptosis).

In addition to tyrosine kinases, protein kinase C (PKC) may mediate TNF-induced K^+ current enhancement (Nietsch et al., 2000). Fas-induced cell shrinkage of Jurkat cells and cellular K^+ loss were blocked by PKC stimulation (Gomez-Angelats et al., 2000). Conversely, inhibition of PKC enhanced the anti-Fas-induced cell shrinkage suggesting an underlying effect of PKC on cellular K^+ loss. PKC exerts an upstream signal in apoptosis and controls the caspase-dependent proteolytic degradation of PKC isotypes. On the other hand, the PKC activator phorbol-12-myristate-13-acetate or bryostatin-1 prevents the activation of caspase-3 and -8. The authors proposed that PKC plays a primary role upstream of activation of caspase-3 and -8 (Gomez-Angelats et al., 2000).

In some peripheral cells, activation of K^+ channels may be responsible for kinase stimulation in apoptosis. The strong enhancement of K^+ currents by UV light irradiation in myeloblastic leukemia (ML-1) cells subsequently activated the JNK/SAPK signaling pathway and resulted in apoptosis (Wang et al., 1999). Suppression of the Kv channel activation

with specific channel blockers prevented UV-induced apoptosis through the inhibition of SEK (SAPK kinase) and JNK activation. Elimination of extracellular Ca^{2+} had no effect on the UV-induced, K^+ channel-mediated JNK/SAPK activation (Wang et al., 1999).

Collectively, these observations are consistent with the notion that protein phosphorylation involving multiple kinases plays critical roles in apoptotic cascades (Anderson, 1997). They also imply a close association between phosphorylation states and the pro-apoptotic regulation of K^+ transport systems.

3.3. Attenuating K^+ efflux and anti-apoptosis effects

Concordant with a key role of K^+ channels in apoptosis, K^+ channel blockers are protective against several forms of apoptosis in many cell types. In addition to the classical K^+ channel blocker TEA or 4-AP, other K^+ channel blockers have been shown to be anti-apoptotic, including the TEA analog tetrapentylammonium (TPeA) and tetrahexylammonium (THA) (Dallaporta et al., 1999; Wang et al., 2000), clofilium (Yu et al., 1999b), quinine (Gantner et al., 1995; Elliott and Higgins, 2003), bretylium tosylate (Nagy et al., 1995), clotrimazole, and charybdotoxin (Pena et al., 2002; Elliott and Higgins, 2003) (Table 3). The K^+ channel blocker TPeA blocked all features of thymocyte apoptosis induced by dexamethasone, etoposide, irradiation, and ceramide. The addition of TPeA prevented cellular K^+ loss, cell shrinkage, mitochondrial disruption, chromatin condensation as well as caspase/endonuclease activation (Dallaporta et al., 1999). The ability of K^+ channel blockers in preventing apoptotic shrinkage was also shown in neurons (Yu et al., 1997; Wang et al., 2000). A recent study showed that K^+ channel blockers might protect hippocampal neurons by reducing microglia-released diffusible messengers such as NO, TNFα, and ROS (Fordyce et al., 2002). In this study, the microglia-released factors in the transwell chambers treated with lipopolysaccharide induced neuronal apoptosis. The co-application of 4-AP (5 mM), AgTx-2 (5 nM), or charybdotoxin (50 nM) in the microglia culture attenuated pro-apoptotic factors and neuronal apoptosis.

In addition to K^+ channel blockers, raising extracellular K^+ typically improves the survival of cultured neurons (Scott and Fisher, 1970; Bennett and White, 1979; Nishi and Berg, 1981; Gallo et al., 1987; Collins et al., 1991) and attenuates neuronal apoptosis induced by growth factor deprivation (Franklin and Johnson Jr., 1992; de Luca et al., 1996), exposure to staurosporine (Yu et al., 1997) or 50% oxygen (Enokido and Hatanaka, 1993). The anti-apoptotic effect of high K^+ medium was initially attributed to its ability to increase Ca^{2+} influx, primarily through voltage-gated Ca^{2+} channels, thus raising $[Ca^{2+}]_i$ towards a "set-point" optimal for survival (Koike et al., 1989; Johnson Jr. et al., 1992). This Ca^{2+} mechanism, however, was excluded from the neuronal protection of high K^+ medium in cortical neurons based on the evidence that: (1) the effect of high K^+ medium cannot be eliminated by blocking voltage-gated Ca^{2+} channels and the increases in $[Ca^{2+}]_i$ (Yu et al., 1997, 1998, 1999b), and (2) high K^+ medium retains its effect in conditions where basal levels of $[Ca^{2+}]_i$ are already elevated by an apoptotic insult such as NMDA (Yu et al., 1999a) or ouabain (Xiao et al., 2002c). These data suggest that an attenuated K^+ efflux due to the diminished K^+ gradient across the membrane is most likely responsible for the anti-apoptotic effect of a high K^+ medium in these cells. Moreover, a recent paper showed that the high K^+ medium required for cerebellar

Table 3
K^+ channel blockers with anti-apoptosis property

K^+ channel blocker and concentration	Cell type	References
Barium (5 mM), TEA (15 mM), glibenclamide (500 μM)	Renal proximal tubules	Reeves and Shah, 1994[a]
4-AP (1–3 mM)	Human eosinophils	Beauvais et al., 1995
Quinidine (10–100 μM), sparteine (3–10 mM)	Human eosinophils; liver cell line	Beauvais et al., 1995; Gantner et al., 1995; Nietsch et al., 2000
TEA (1–5 or 10–20 mM) and active analogs (TPeA, THA, low μM)[b]	Central neurons, cholinergic septal cell lines	Yu et al., 1997, 1998, 1999b; Colom et al., 1998; McLaughlin et al., 2001
Clofilium (low μM)	Cortical neurons, porcine granulosa cell	Yu et al., 1999b; Manikkam et al., 2002
Barium (0.1 mM), quinine (0.1 mM)	Liver cell line (HTC)	Nietsch et al., 2000
TEA (1 mM) and iberiotoxin (IBTX, 100 nM)	Pulmonary vascular smooth muscle cells	Krick et al., 2001
Quinine (0.5 mM), clotrimazole (10 μM), charybdotoxin (200 nM)	T lymphocytes	Elliott and Higgins, 2003

4-AP and clofilium alone may induce apoptosis. The mechanism for their pro-apoptotic action is elusive. Recent evidence suggests that a potent inhibitory effect of these compounds on Na^+, K^+-ATPase may be responsible for the induction of apoptosis (Xiao et al., 2002a; Yang et al., 2002a,b; Wang et al., 2003a,b).

[a] The type of cell death was not defined in this study except that DNA damage was assessed.

[b] TPeA: tetrapentylammonium; THA: tetrahexylammonium.

granule cultures blocked the pro-apoptotic effect of cytosine arabinose usually used in the culture procedures; without cytosine arabinose, high K^+ was not required for these cells to survive (Daniels and Brown, 2002). Therefore, it is possible that some previous studies on granule cultures might actually investigate the cytotoxicity of cytosine arabinose.

Conflicting with the data showing the anti-apoptotic effect of K^+ channel blockers, a few reports have shown that K^+ channel blockers 4-AP and clofilium induce apoptosis in malignant astrocytoma cell lines (Chin et al., 1997), human promyelocytic leukemia (HL-60) cells (Choi et al., 1999), human hepatoblastoma cells (Kim et al., 2000), and porcine granulose cells (Manikkam et al., 2002). A recent study also showed that K^+ channel blockers, dequalinium, amiodarone, and glibenclamide caused apoptosis in human prostate cancer cell lines through an undefined mechanism (Abdul and Hoosein, 2002). Whether these toxic effects are due to blocking the K^+ channels or a result from non-specific actions of these compounds remains to be determined. Our recent studies show that the cell death induced by 4-AP and clofilium is likely a result from their potent inhibitory actions on the Na^+, K^+-ATPase at similar concentrations that inhibit K^+ channels (Yang et al., 2002a,b; Xiao et al., 2002a; Wang et al., 2003a,b).

4. Mitochondria and K^+ regulation of apoptosis

4.1. Mitochondrial K^+ homeostasis and apoptosis

The relationship between mitochondrial dysfunction and apoptosis has been reviewed previously (Jacotot et al., 1999; Crompton, 1999). In this review, the author evaluates a few issues that have not been emphasized before.

Induction of apoptosis by valinomycin may not only involve augmented K^+ efflux into the extracellular space but also be associated with the disruption of the K^+ gradient across the mitochondrial membrane. Since the outer surface of valinomycin is hydrophobic, the molecule, which is soluble in lipid bilayers, can diffuse across the membranes. When added to intact cells, valinomycin can transport K^+ ions both out across the plasma membrane and in across the mitochondrial membrane, driven by the respective K^+ gradients (Fig. 1). In fact, valinomycin is well known for causing depolarization and the collapse of the inner mitochondrial membrane potential ($\Delta\Psi$), an effect correlated to DNA fragmentation (Ojcius et al., 1991; Inai et al., 1997; Dallaporta et al., 1998; Bortner and Cidlowski, 1999). Valinomycin decreases the $\Delta\Psi$ likely by collapsing the K^+ gradient across the mitochondrial inner membrane (Kovac et al., 1982; Inai et al., 1997; Scorrano et al., 1997). Simultaneous measurements of the mitochondrial and plasma membrane potentials in several cultured cell lines showed that valinomycin induced rapid (in minutes) depolarization of the mitochondria and the cell, as a results from K^+ fluxes into the mitochondria and the extracellular space (Farkas et al., 1989).

A recent novel study further revealed a close relationship between mitochondrial K^+ homeostasis and apoptosis. Eliseev et al. (2003) showed in HL-60 cell lines that the apoptotic insult etoposide induced an enhanced mitochondrial K^+ uptake (e.g. influx via the mK_{ATP} channel) preceding cytochrome *c* release, an event associated with a transient K^+ accumulation and swelling of mitochondria. Overexpression of *bcl-2* in HL-60 cells or adding recombinant *bcl-2* protein to the isolated rat liver mitochondria inhibited the K^+ influx. Stimulating the K^+ influx using the K_{ATP} channel opener diazoxide either in HL-60 cells or the isolated mitochondria resulted in cytochrome *c* release and caspase-3 activation, and all events were blocked by the specific channel blocker 5-hydroxydecanoate as well as by overexpression of *bcl-2*. In searching for the protective mechanism by *bcl-2*, the study showed that *bcl-2* increased removal of K^+ from mitochondria via the mitochondrial K/H-exchanger. The N-terminal truncated *Bid* that had increased ability to translocate into mitochondria drastically increased the mitochondrial K^+ uptake and cytochrome *c* release in a *bcl-2*-sensitive manner. Thus, to a great extent *bcl-2* and *Bid* may regulate mitochondrial damage and cytochrome *c* release by controlling the mitochondrial K^+ homeostasis (Eliseev et al., 2003).

4.2. Mitochondrial membrane depolarization and K^+ permeable channels

Permeability transition (PT) is the opening of protaceous pores in the inner mitochondrial membrane allowing free distributions of solutes between matrix and cytosol (Bernardi, 1999). Isolated mitochondria undergoing PT can induce apoptotic events in nuclei in a cell free system, suggesting that PT may be a critical event in activating late stages of apoptosis (Marchetti et al., 1996). The $\Delta\Psi$ decrease induced by K^+ flux into the matrix may be responsible for PT (Bernardi et al., 1992). The increase in K^+ permeability of inner mitochondrial membrane may also be responsible for an increased permeability of mitochondria to protons, leading to intracellular acidification (Balakirev and Zimmer, 1998; Furlong et al., 1998). Supporting a close relevance to apoptosis, changes in the mitochondrial membrane potential, along with several other characteristics of apoptosis, appear to be restricted only to the shrunken population of cells (Bortner and Cidlowski, 1999). The decrease in $\Delta\Psi$ has been recognized during apoptosis in a number of cells triggered by different insults (Furlong et al., 1998; Kroemer and Reed, 2000). Activation of caspase-3 occurs after depolarization of the mitochondria (Inai et al., 1997), which is consistent with the mitochondria related events of cytochrome *c* release, Apaf-1 release, and formation of apoptosomes, all occurring before caspase-3 activation (Adams and Cory, 2002) (Fig. 2).

A recent study supported a role for a Ca^{2+}-activated K^+ channel in mitochondria membrane permeability increase and neuronal death; blocking the mitochondrial channel with charybdotoxin significantly delayed cell death in the absence

of trophic support (Pena et al., 2002). A highly K^+ permeable channel protein closed by ATP and glibenclamide named mK_{ATP} channel was identified in the inner membrane of the mitochondria of liver, heart, and recently in neurons (Diwan et al., 1988; Inoue et al., 1991; Paucek et al., 1992; Bajgar et al., 2001). The mK_{ATP} channel shows similar properties as the K_{ATP} channel in the plasma membrane. Activation of the mK_{ATP} channel leads to a flux of K^+ from the cytosol into the mitochondria, consequently causing depolarization of the $\Delta\Psi$ (Kowaltowski et al., 2001; Bajgar et al., 2001), increase of matrix pH (Garlid and Paucek, 2001), increase of the matrix volume (Garlid and Paucek, 2001; Kowaltowski et al., 2001), decrease of the intermembrane space (Kowaltowski et al., 2001), changes in permeability of the voltage-dependent anion channel (VDAC) of the outer mitochondrial membrane to nucleotides (Garlid and Paucek, 2001), and induction of ROS (Obata and Yamanaka, 2000; Garlid and Paucek, 2001; Forbes et al., 2001). These events are apparently insults to the cell and may serve as a mechanism for the pre-conditioning effect of K_{ATP} channel openers (see Section 7.2 and Garlid et al., 1996; Liu et al., 1999; Ghosh et al., 2000; Dos Santos et al., 2002; Rajapakse et al., 2002).

5. Ionotropic glutamate receptor channels and pro-apoptotic K^+ efflux

5.1. K^+ permeability of ionotropic glutamate receptor channels

L-Glutamate acts as a major excitatory neurotransmitter in the CNS (Watkins and Evans, 1981; Mayer and Westbrook, 1987a; Monaghan et al., 1989; Hollmann and Heinemann, 1994). Over-activation of ionotropic glutamate receptors (NMDA, AMPA/kainite receptors) may cause neuronal death after brain injury, such as ischemia, head trauma, epileptic seizures, as well as in neurodegenerative diseases (Schwarcz and Meldrum, 1985; Rothman and Olney, 1986; Choi, 1988b, 1992). Excessive Ca^{2+} and Na^+ influx has been linked to glutamate, NMDA, and AMPA/kainate receptor-induced excitotoxicity in the CNS (Choi, 1988a, 1992; Olney, 1990; Sattler and Tymianski, 2001). The cell death produced by glutamate and glutamate analogs can produce either necrosis or apoptosis, in vitro as well as in vivo (Ankarcrona et al., 1995; van Lookeren Campagne et al., 1995; Du et al., 1996; Qin et al., 1996; Kawasaki et al., 1997; Lesort et al., 1997; Portera-Cailliau et al., 1997; Tenneti et al., 1998; Budd and Lipton, 1999; Gary and Mattson, 2001), yet the mechanism of glutamate receptor activation-induced apoptosis is not well defined.

In addition to Ca^{2+} and Na^+, ionotropic glutamate receptor channels are highly permeable to K^+ (MacDermott et al., 1986; Mayer and Westbrook, 1987b; Ascher and Nowak, 1988; Iino et al., 1990). In fact, L-glutamate-stimulated K^+ efflux was observed years before the identification of the Na^+ and Ca^{2+} permeabilities of glutamate receptors (Eggleston and Krebs, 1950; Harvey, 1968; Anwyl, 1977; Teichberg et al., 1981; MacDermott et al., 1986; Mayer and Westbrook, 1987b; Ascher and Nowak, 1988). Eggleston and Krebs (1950) were the first to report that after 1 h incubation L-glutamate led to an increased extracellular content of K^+ in cerebral tissues in vitro. Later, it was confirmed that addition of L-glutamate or NMDA rapidly stimulated cellular K^+ loss, the extracellular K^+ increased up to 60 mM in just a few minutes (Harvey, 1968; Sonnhof et al., 1975; Buhrle and Sonnhof, 1983). A number of studies showed that NMDA receptors have at least an equal, if not higher, permeability to K^+ as to Na^+ (Jan and Jan, 1976; Anwyl, 1977; Matthews and Wickelgren, 1979; Dekin, 1983; Mayer and Westbrook, 1987a; Ascher et al., 1988; Ozawa et al., 1988; Chang et al., 1994; Tsuzuki et al., 1994), though their permeability to Ca^{2+} is several folds higher than to Na^+ or K^+ (Mayer and Westbrook, 1987a; Ozawa et al., 1988; Chang et al., 1994). Even though the glutamate receptor K^+ permeability has been known for many years, little attention has been given to the K^+ movement. This has been ignored to the extent that it is often forgotten that the K^+ efflux through glutamate/NMDA receptor channels occurs concurrently with Ca^{2+} and Na^+ influx at similar membrane potentials. This simultaneous two-way traffic is evidenced by the fact that NMDA currents normally have a reversal potential of around 0 mV and supported from calculated *I–V* relations based on equilibrium potentials of Ca^{2+}, Na^+, and K^+ (Mayer and Westbrook, 1987a).

5.2. Ionotropic glutamate receptor channels-mediated K^+ efflux and apoptosis

We hypothesized a few years ago that under certain pathological conditions where the glutamate receptor-mediated K^+ efflux and intracellular K^+ depletion became prominent then stimulation of these receptors would cause substantial apoptosis in glutamate/NMDA excitotoxicity. To demonstrate this possibility, we identified the NMDA outward current mediated by K^+ efflux at negative and positive membrane potentials (Yu et al., 1999a). Cortical neurons exposed to NMDA or endogenously released glutamate in a medium containing reduced Na^+ and Ca^{2+} (as found in ischemic brain tissues) showed cell body shrinkage, loss of about 50% of intracellular K^+, and other apoptotic features such as DNA laddering and sensitive to caspase inhibition. Attenuating K^+ efflux by increasing extracellular K^+ attenuated both the K^+ loss and apoptosis (Yu et al., 1999a). Similarly, stimulating the AMPA receptor in normal and the low Ca^{2+}/low Na^+ medium, respectively, induced about 60 and 80% cellular K^+ losses. In this study, cyclothiazide was added to prevent desensitization of AMPA receptors. As expected, the apoptotic component was more apparent in the low Ca^{2+}/low Na^+ solution shown as caspase activation, cell body shrinkage, and attenuated cell death in the presence of a caspase inhibitor or protein synthesis inhibitor

(Xiao et al., 2001). The K^+ depletion-induced apoptosis was also observed with activation of kainate receptors (Xiao et al., 2001). These investigations illustrate that the K^+ permeability of ionotropic glutamate receptors may have significant impacts on K^+ homeostasis and induction of apoptosis. Of note, even under the low Ca^{2+}/low Na^+ condition apoptosis induced by NMDA or non-NMDA receptor activation accounts for only part (~20–50%) of excitotoxicity (Yu et al., 1999a; Xiao et al., 2001), suggesting a mixed form of cell injury (see Section 6.2 for further discussion).

5.3. High homology between ionotropic glutamate receptor channels and voltage-gated K^+ channels: functional implications

Upon recognizing the putative important role of ionotropic glutamate receptor-mediated K^+ efflux in apoptosis, we proposed that regulation of the K^+ permeability of these receptors deserved special attention (Yu and Ichinose, 1999; Ichinose and Yu, 2000; Ichinose et al., 2003a,b). The conventional idea believes that ion permeabilities of glutamate receptors are uniformly regulated. This assumption has been challenged by reports that the Ca^{2+} permeability can be individually changed (Burnashev et al., 1992; Schneggenburger, 1998). An evidence making the glutamate receptor K^+ permeability unique is the recent discovery of a striking degree of amino acid homology between the pore-forming regions of voltage-gated K^+ channels and ionotropic glutamate receptors (Wo and Oswald, 1995; Wood et al., 1995; Tikhonov et al., 1999). A common C-terminal binding motif of NMDA receptor NR2A, NR2B, and *Shaker* K^+ channels for PDZ domains of PSD-95/SAP90 family of membrane-associated guanylate kinases suggests similarities between the C-termini of NMDA receptor NR2 subunits and K^+ channels (Niethammer et al., 1996). The idea of structural conservation between K^+ channels and glutamate receptor channels is strongly supported by the discovery of the novel GluR0 receptor (Chen et al., 1999). GluR0, found in prokaryotic cells, binds glutamate and forms K^+-selective channels; it is related in amino-acid sequence to both eukaryotic ionotropic glutamate receptor channels and K^+ channels. It is proposed that GluR0 is the evolutionary precursor to eukaryotic ionotropic glutamate receptors, providing the missing link between K^+ channels and ionotropic glutamate receptors. The authors conclude that the M1, P and M2 pore-forming regions in GluRs and K^+ channels have very similar architecture, and these two channels share some essential features in gating mechanisms (Chen et al., 1999). The other ligand-gated channels, including the acetylcholine, GABA, glycine, and serotonin 5HT3 receptors, show much weaker homology with K^+ channels (Wood et al., 1995), suggesting the selectivity of this homology.

Although the K^+ permeability of NMDA receptors has its inherited root in voltage-gated K^+ channels, there was little understanding whether this homology may indicate functional similarities. Our recent investigations reveal that the K^+ efflux mediated by NMDA receptors is likely under a Ca^{2+}-independent but voltage/activity-dependent regulation different from the regulation of Ca^{2+} and Na^+ influx (Yu and Ichinose, 1999; Ichinose and Yu, 2000; Ichinose et al., 2003a,b). Specifically, the NMDA receptor mediated outward NMDA-K current is not subject to Ca^{2+}-dependent rundown, which is a well known event for the NMDA inward current carried by Ca^{2+} and Na^+ influx. Moreover, pre-existing membrane depolarization and membrane excitatory activities exhibit a selective up-regulation of the NMDA-K current (Ichinose et al., 2003a,b). We propose this selective regulation of the NMDA receptor permeability may play a unique role in physiological and pathological conditions and deserves further investigation.

6. Na^+, K^+-ATPase and apoptosis

6.1. Na^+, K^+-ATPase and K^+ homeostasis

The Na^+, K^+-ATPase (Na^+, K^+ pump) plays a critical role in maintaining ionic homeostasis as well as other cellular functions, acting as the primary system to move extracellular K^+ back into cells and move intracellular Na^+ out into extracellular space against their respective concentration gradients across the membrane (Gloor, 1997; Schmidt and Kjeldsen, 1997). Theoretically, K^+ homeostasis will not be disrupted as long as K^+ efflux can be balanced by a sufficient K^+ uptake. Since the Na^+, K^+-ATPase is the only major mechanism for K^+ uptake, its activity is likely a decisive factor in controlling K^+ homeostasis and induction of apoptosis. Based on this view, we argued recently that in order for cells to lose a significant amount of intracellular K^+, in addition to the enhanced K^+ efflux via K^+ permeable channels, cells undergoing apoptosis might concurrently suffer from a dysfunction of the Na^+, K^+-ATPase. Supporting this hypothesis, recent studies have suggested that although some apoptotic processes such as formation of the apoptosome depend on the presence of some minimal level of ATP, mitochondrial metabolism and ATP level in apoptotic cells are progressively and significantly lower than that in normal cells (Nord et al., 1997; Feldenberg et al., 1999; Latta et al., 2000; Komatsu et al., 2000; Yang et al., 2002a,b). In our investigation, we have directly demonstrated for the first time that the Na^+, K^+-ATPase activity measured as a membrane current I_{pump} in cortical neurons was time-dependently suppressed by apoptotic insults including serum deprivation, staurosporine, and C_2-ceramide, concomitant with depletion of intracellular ATP and production of ROS. The apoptosis-associated Na^+, K^+-ATPase failure and serum deprivation-induced neuronal death were antagonized by pyruvate and succinate in ATP- and ROS-dependent manners (Wang et al., 2003a,b).

Failure of the Na^+, K^+-pump results in depletion of intracellular K^+, accumulation of intracellular Na^+, and, consequently, leads to membrane depolarization and increases

in $[Ca^{2+}]_i$ due to activation of voltage-gated Ca^{2+} channels and a reversed operation of the Na^+–Ca^{2+} exchanger (Archibald and White, 1974; Lijnen et al., 1986; DiPolo and Beauge, 1991; Xiao et al., 2002c). Ouabain, the selective inhibitor of the Na^+, K^+ ATPase, may cause apoptosis in different cell types (Olej et al., 1998; Verheye-Dua and Bohm, 2000; Mann et al., 2001). The mechanisms underlying the ouabain-induced apoptosis were elusive, although Ca^{2+} influx and K^+ efflux have been proposed to mediate the ouabain effect in human adenocarcinoma cells (McConkey et al., 2000). We tested the hypothesis in cultured cortical neurons that a K^+ loss might be an essential contributor to ouabain toxicity especially ouabain-induced apoptosis. Ouabain (80–100 μM) caused cell swelling in minutes as previously reported (Strange, 1990; Jurkowitz-Alexander et al., 1992). However, long-term observation revealed that the cell swelling was followed by gradual cell shrinkage in the next 24 h accompanied by 80% of intracellular K^+ depletion (Xiao et al., 2002c). It is necessary to note that such a long time period for shrinkage could not be explained by the mechanism of regulatory volume decrease (RVD), which typically lasts for several minutes (Guizouarn et al., 2000). Ouabain-induced cell death is partially prevented by blocking caspases, implying an apoptotic component in ouabain toxicity. Moreover, the K^+ channel blocker TEA attenuated ouabain-induced cellular K^+ loss, cell shrinkage, and apoptotic death, confirming that K^+ left the cell via K^+ channels and K^+ loss played a key role in ouabain-induced apoptosis (Xiao et al., 2002c).

We showed recently that even a slight impairment of the pump activity could synergistically aggravate ceramide- and β-amyloid-induced apoptosis in cortical neurons (Xiao et al., 2002b). A low concentration of ouabain (0.1 μM), which marginally inhibited the pump current and showed no toxicity, triggered marked cellular K^+ depletion, caspase activation, DNA laddering, and apoptotic death when it was combined with sub-lethal C_2-ceramide and β-amyloid 1–42 (Xiao et al., 2002b). This observation may be clinically important under conditions where slight dysfunction of the Na^+, K^+-ATPase and a sub-lethal apoptotic insult could take place concurrently.

6.2. Na^+, K^+-ATPase and hybrid cell death

As mentioned above, blocking the Na^+, K^+-ATPase not only reduces intracellular K^+ but also increases intracellular Ca^{2+}; the latter event is believed a trigger for excitotoxicity of necrotic cell death (Choi, 1988a). Interestingly, under the condition of severe block of the Na^+, K^+-ATPase by 80–100 μM ouabain, we identified that both necrotic and apoptotic features coexist in individual neurons, in agreement with the concomitant Ca^{2+} accumulation and K^+ depletion in these cells (Xiao et al., 2002c). This mixed cell death is identified as a distinct form of 'hybrid death' to distinguish it from 'pure' necrosis and 'pure' apoptosis. The hybrid cell death possesses characteristics of apoptosis such as caspase activation, DNA fragmentation, and morphological changes of shrunken nuclei and chromatin condensation; meanwhile, necrotic changes are apparent in the same cells including deteriorated membranes and chaotic disruption of the cytoplasm and cellular organelles (Xiao et al., 2002c). These findings may have significant clinical relevance as endogenous ouabain-like substances or hypothalamic digoxin increase in a number of disease states leading to inhibition of Na^+, K^+-ATPase (Kumar and Kurup, 2002). This view is supported by increasing reports showing mixed forms of cell death in pathological conditions (Portera-Cailliau et al., 1997; Warny and Kelly, 1999; Takashi and Ashraf, 2000; Nakajima et al., 2000), dictating reassessment of 'mixed cell death' as a heterogeneous entity combining both active and passive cell death (Hirsch et al., 1997; Kim et al., 1999; Yu et al., 1999a; Xiao et al., 2002c) and in line with an in vivo "apoptosis–necrosis continuum" in excitotoxically lesioned rat brain (Portera-Cailliau et al., 1997).

7. Clinical implication of the K^+ regulation of apoptosis

7.1. Modulators of K^+ homeostasis as potential therapeutic drugs

Potassium channel blockers have been proposed as a therapeutic strategy in patients with Alzheimer's disease and brought into clinical trials (Wesseling et al., 1984; Lavretsky and Jarvik, 1992), because of the potential of improving acetylcholine release. K^+ channel blockers have also been used in treatment of cardiovascular diseases such as arrhythmia (Duff et al., 1991). Some K^+ channel blockers like 4-AP have been investigated as beneficial symptomatic treatments for a variety of neurological conditions of demyelination diseases including multiple sclerosis, myasthenia gravis, and spinal cord injury (Murray and Newsom-Davis, 1981; Jones et al., 1983; Targ and Kocsis, 1985; van Diemen et al., 1992; Blight et al., 1991; Hayes et al., 1993; Hansebout et al., 1993; Waxman, 1993; Segal and Brunnemann, 1997; Potter et al., 1998; Halter et al., 2000). Clofilium, the potent class III anti-arrhythmic drug, has been used in animals and humans for the treatment of ventricular tachycardia (Greene et al., 1983; Platia and Reid, 1984; Kopia et al., 1985) and atrial fibrillation (Borzak et al., 1997).

The K^+ mechanism underlying apoptosis and the anti-apoptotic effects of K^+ channel blockers provide new therapeutic approaches to apoptosis-related disorders. TEA and clofilium showed the protective effect of reducing infarct volume after global and focal brain ischemia in animal models (Choi et al., 1998; Huang et al., 2001; Wei et al., 2003), which is consistent with the observation that hypoxia/anoxia or ischemia/reperfusion may activate various K^+ currents in central neurons (Cowan and Martin, 1992; Jiang and Haddad, 1993; Haddad, 1997; Guatteo et al.,

1998; Mironov and Richter, 2000; Xuan and Xu, 2000). K^+ channel activation and the protective effects of TEA and glibenclamide were also observed after a hypoxic insult to renal proximal tubules (Reeves and Shah, 1994). K^+ channel alterations may be associated with the membrane depolarization in cells from scrapie-infected mice, and this depolarization can be prevented by TEA, providing evidence that the scrapie-related membrane depolarization and some pathological changes may result from activation of K^+ channels and loss of intracellular K^+. It is possible that altered K^+ channels might be a pathological mechanism triggering neurodegenerations in scrapie-related diseases and that these channels might be targeted as a therapeutic approach (Johnston et al., 1998).

Because blocking K^+ channels induces membrane depolarization and increases in $[Ca^{2+}]_i$, confounding side effects of excitotoxicity have been a major consideration for clinical uses of these drugs. Being that relatively lower concentrations of K^+ channel blockers (~30% channel block) are needed to prevent the pro-apoptotic excessive K^+ efflux, the influence on the normal activity of K^+ channels and side effects at therapeutic dosages could be minimum (Choi et al., 1998; Wei et al., 2003). In fact, the toxic action of K^+ channel blockers may often come from their non-specific actions on other channels or transporters, particularly the Na^+, K^+-ATPase. For example, TEA may voltage-dependently block the Na^+, K^+-ATPase by a direct interference with the extracellular K^+ binding site of the pump protein (Eckstein-Ludwig et al., 1998). Among K^+ channel blockers, 4-AP is well known for its high neurotoxicity at relatively low concentrations. At similar concentrations, we recently demonstrated a potent inhibitory effect of 4-AP on Na^+, K^+-ATPase (Xiao et al., 2002a). These observations call for the development of more selective K^+ channel blockers with the potential of improving anti-apoptosis efficacy while reducing side effects.

An alternative anti-apoptotic approach is to enhance K^+ uptake. Our recent data show that pyruvate and succinate attenuate apoptosis by preserving the Na^+, K^+-ATPase activity (Xiao et al., 2002c). A number of means can positively regulate the Na^+, K^+-pump activity, including increased energy supply, phosphorylation, anti-oxidative agents, and drugs/hormones that augment the pump activity (Clausen, 1996; Cornelius et al., 2001; Lopina, 2001; Pedemont and Bertorello, 2001). Application of these and other maneuvers for improving the Na^+, K^+-pump function and attenuating apoptosis may prove to be an effective antagonism against apoptosis.

Since ionotropic glutamate receptors may contribute to pro-apoptotic K^+ efflux, means of reducing activation of these receptors or that of selectively preventing the K^+ efflux should attenuate apoptosis. Based on the recognition that the hybrid cell death may occur in pathological conditions in vivo, a combination of therapeutic approaches appears necessary to target both necrosis and apoptosis. In this case, preventing intracellular K^+ depletion may be considered an essential complementary means of treatment.

7.2. K^+ channel openers and blockers: mechanism of protection

In the last few years, a class of drugs with the property of opening the K_{ATP} channels in the plasma membrane or mitochondrial membrane has been shown to be protective against ischemic injury in the heart (Kitamura and Kamouchi, 1993; Edwards and Weston, 1995; Liu et al., 1998; Gross and Fryer, 1999; Grover and Garlid, 2000; Akao et al., 2001; Dos Santos et al., 2002) as well as brain (Murphy and Greenfield, 1991; Rajapakse et al., 2002). Similarly, opening of the Ca^{2+}-sensitive maxi-K channel was proposed as a therapeutic treatment of ischemic stroke (Gribkoff et al., 2001), although clinical trials were disappointing (Goldberg, 2002). K^+ channel openers can hyperpolarize the membrane as long as K^+ homeostasis remains relatively normal. With a hyperpolarized membrane potential, voltage-dependent Ca^{2+} and Na^+ influx will be eliminated or attenuated and thus prevent ischemia-induced necrotic cell death in the heart (Grover, 1997; Garlid and Paucek, 2001) or brain (Abele and Miller, 1990; Fujita et al., 1997; Takaba et al., 1997). In addition, the K_{ATP} channel opener cromakalim attenuated glutamate-induced apoptosis in hippocampal neurons. The effect was linked to an inhibition of the delayed increase in $[Ca^{2+}]_i$ (Lauritzen et al., 1997).

The mechanisms by which K^+ channel openers reduce cell death, however, are debatable. K^+ channel openers such as pinacidil may interfere with Ca^{2+} mobilization from intracellular stores (Itoh et al., 1992). These openers including diazoxide, levocromakalim, and pinacidil may also act as antioxidants; they were effective in protecting neurons against oxidative insults in the presence of the K^+ channel blockers glibenclimide and 4-AP indicating that their protective mechanism involved actions in addition to activation of K^+ channels (Goodman and Mattson, 1996). The involvement of K_{ATP} channels in the observed effects has been questioned based on the following arguments: (1) the concentrations for cromakalim and other K^+ channel openers to cause the effect (e.g. vasodilation or neuroprotection) can be many times lower or higher than that required to open the K^+ channel (Quast, 1993; Goodman and Mattson, 1996), and (2) the observed protective effects of K_{ATP} channel openers may not be antagonized by a channel blocker selectively targeting the K_{ATP} channel (Quast, 1993; Goodman and Mattson, 1996). A pre-treatment of these K_{ATP} channel openers is usually required for the protective effect, suggesting that they likely trigger a pre-conditioning mechanism (Mizumura et al., 1995; Menasche et al., 1995). In other words, the K^+ channel openers themselves may generate an insult, which is consistent with the K^+ mechanism underlying apoptosis. Promoting K^+ efflux via the plasmalemmal K_{ATP} channel and various consequences in the mitochondria following

opening the mK_{ATP} channel may serve as mediators of the pre-conditioning effect (see Section 4.2 for effects on mitochondrial functions). This opinion is supported by a recent report that diazoxide pre-treatment induced a transient and mild depolarization of the mitochondrial membrane potential (Ichinose et al., 2003a,b). K^+ channel openers may therefore protect cells via a pre-conditioning effect or hyperpolarizing the membrane provided K^+ homeostasis has not been significantly altered. In the event that $[K^+]_i$ has already been decreased, opening K^+ channels will only further depolarize the membrane due to the positively shifted K^+ equilibrium potential and additional K^+ efflux. Other mechanisms not associated with a pre-conditioning effect and membrane hyperpolarization may be possible but remain to be specifically substantiated. Interestingly, in a coronary occlusion–reperfusion animal model, both opening and blocking K_{ATP} channels increased survival rate (Baczko et al., 1997), thus K^+ channel modulators may be beneficial via different or even opposing mechanisms.

7.3. The K^+ mechanism underlying apoptosis and potential cancer therapy

In addition to anti-apoptotic applications, the understanding of the role of K^+ in apoptosis may add new strategies for the treatment of cancer and other diseases. K^+ channels play critical roles in cell proliferation including cancer cells (Schell et al., 1987; Knutson et al., 1997; Gallagher et al., 1996; Wang et al., 1997; Rouzaire-Dubois and Dubois, 1998). The human ether-a-go-go related gene (*HERG*) encodes a K^+ channel that facilitates tumor cell proliferation and markedly promotes H_2O_2-induced apoptosis of various tumor cells (Wang et al., 2002). Recent evidence has shown that stimulating K^+ efflux by the K^+ channel modulatory protein KchAP/PIAS3β induces or enhances apoptosis in cancer cells (Wible et al., 2002). Supporting that K^+ channels and K^+ efflux may regulate carcinogenesis, Coma et al. (2003) showed that Kv channel expression was impaired in the brain of tumor-bearing animals. Expression of both delayed rectifier (Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv1.6, Kv2.1, Kv3.1, Kv4.2) and A-type K^+ channels (Kv1.4, Kv3.3, Kv3.4) was greatly down-regulated in the brain from animals bearing a Yoshida AH-130 ascites hepatoma. Further study will be interesting and necessary to evaluate the impaired K^+ channel expression and K^+ channel function in other cancer cells. Tested in the pulmonary mesothelioma cell line (P31), caspase activation and apoptosis induced by the anti-cancer drugs cisplatin or carboplatin was enhanced by stimulating K^+ efflux using the K^+ ionophore amphotericin B and/or the Na^+, K^+, $2Cl^-$ cotransporter blocker bumetanide. It was suggested that drugs stimulating K^+ efflux might be considered alone or in combination for their potential clinical use in influencing tumor cell apoptosis induced by cancer chemotherapeutics (Marklund et al., 2001b). Using the K^+ analogue $^{86}Rb^+$ as the probe for K^+ fluxes, these authors confirmed that amphotericin B and bumetanide indeed stimulated K^+ efflux and/or prevented K^+ influx (Marklund et al., 2001a).

8. Final remarks

In addition to the genetic regulation of apoptosis, increasing evidence now endorses the idea of a K^+ based ionic mechanism for apoptosis. The study of the K^+ mechanism for apoptosis provides an exciting opportunity for a more complete understanding of the cellular program for death and survival. Although substantial evidence has been accumulated, a number of important issues call for further investigations.

(1) What are possible interactions between K^+ and other ions in the apoptotic process? Ca^{2+} is likely a regulator in the apoptotic process involving protease activity, DNA fragmentation, mitochondrial damage, as well as other Ca^{2+}-dependent events critical for cell death. Whether K^+ and Ca^{2+} may mutually interact in these events is unclear. Other divalent cations such as Zn^{2+} and Mg^{2+} may also contribute to regulation of apoptosis (Sunderman Jr., 1995; Patel et al., 1994; Yoshida et al., 1998; Chien et al., 1999; Pereira et al., 2002). In addition to cations, Cl^- movement is closely associated with K^+ movement during apoptosis (Maeno et al., 2000), the exact relationship between K^+ and Cl^- homeostasis is not well defined. Moreover, in some cells such as N1E neuroblastoma cells Cl^- movement may play a more important role in apoptosis (O'Reilly et al., 2002) and deserves further investigation.

(2) Are there cell specificities for the ionic mechanism of apoptosis? For example, apoptosis in the cerebellar granule cells or sympathetic ganglion neurons appears primarily under the influence of Ca^{2+} (Koike et al., 1989; Johnson Jr. et al., 1992), but less affected by the K^+ mechanism (Xia et al., 2002).

(3) Astrocytes and oligodendrocytes play important roles in K^+ homeostasis (Hertz, 1992; Dobretsov and Stimers, 1996; Chvatal et al., 1999); it is still unexplored how these non-neuronal cells may participate in the K^+ regulation of neuronal apoptosis. For example, the Na^+, K^+-ATPase and other transporters in non-neuronal cells have significant impacts on K^+ and Na^+ homeostasis (Dobretsov and Stimers, 1996; Rose and Ransom, 1996) and may thereafter influence apoptosis and neuron survival.

(4) What are the signal transduction systems that mediate the pro-apoptotic modulation of K^+ channels? The tyrosine kinase family is likely involved in the I_K channel modulation; specific kinases and other mediators remain to be identified.

(5) Are there any other apoptotic-related proteins controlled or regulated by intracellular K^+? Besides caspases and endonucleases, K^+ may influence activities

of other enzymes, intracellular signals, and even protein synthesis (Ledbetter and Lubin, 1977; Cahn and Lubin, 1978). Information about the relationship of these effects to apoptosis is so far not available.

(6) How may apoptotic genes such as those in the *bcl-2* family affect or can be affected by K^+ homeostasis? The excellent study by Eliseev et al. (2003) has addressed this issue showing the counter-regulation of mitochondrial K^+ transport by *bcl-2* and *Bid*. Additionally, *bcl-2* genes form channels that are highly permeable to K^+ and other ions (Muchmore et al., 1996; Minn et al., 1997; Antonsson et al., 1997; Schlesinger et al., 1997), but the significance of the K^+ permeability is obscure.

(7) It is known that K^+ flux can affect the mitochondrial membrane potential and mitochondrial volume, yet the subcellular mechanism is still ambiguous. For instance, what is the exact relationship between K^+ homeostasis, mitochondrial function, and cytochrome *c* release?

(8) K^+ channel family is composed of a large number of subtypes of K^+ channels; the type of K^+ channels contributing to the pro-apoptotic K^+ efflux may differ in different cells. Whether specific K^+ channel expression and gene mutation may affect induction of apoptosis and pathogenesis remains to be further clarified.

(9) Selective K^+ channel blockers are critical tools in future investigations on the K^+ regulation of apoptosis and for pre-clinical studies. Available K^+ channel blockers often have non-specific actions on other channels and/or transporters especially the Na^+, K^+-ATPase, which likely cause or exaggerate undesirable consequences. Depending on the identification of involved K^+ channels in diseases, K^+ channel blockers with higher selectivity may provide a means of treatment with fewer side effects.

(10) Techniques for accurate measurements of intracellular K^+ concentrations need to be developed in order to delineate some key parameters such as the threshold level of intracellular K^+ for triggering apoptotic cascade. In this respect, more specific K^+ fluorescent dyes will greatly facilitate the research on the relationship between K^+ homeostasis and apoptosis.

(11) In oocytes and granulosa cells, K^+-independent nucleases may exist (Perez et al., 2000), and it will be important to identify and characterize K^+-dependent and –independent apoptotic events in future investigations.

(12) In addition to K^+ channels, ionotropic glutamate receptors, and the Na^+, K^+-ATPase, how may other membrane channels and transporters directly or indirectly influence K^+ homeostasis and apoptosis? Other possible players may include voltage-gated Na^+ channels, Ca^{2+} channels, and ligand-, voltage-gated Cl^- channels.

(13) The ionic mechanism underlying apoptosis may not be specific to K^+; in the studies on lymphocytes, monovalent cations such as Na^+, Cs^+, Li^+, Cd^+ and anion Cl^- all were effective in blocking DNA degradation, suggesting that the ionic strength might be relevant to the DNA damage (Hughes Jr. et al., 1997). Whether this is true for other apoptotic events in other cell types remains to be tested.

We are at the early stage of understanding the relationship between K^+ homeostasis and apoptosis. It can be predicted that continuous investigations in this exciting area will help us to decode more information in the cell death program and may delineate possible implications and therapeutic treatments in clinical settings.

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References

Abdul, M., Hoosein, N., 2002. Expression and activity of potassium ion channels in human prostate cancer. Cancer Lett. 186, 99.

Abele, A.E., Miller, R.J., 1990. Potassium channel activators abolish excitotoxicity in cultured hippocampal pyramidal neurons. Neurosci. Lett. 115, 195–200.

Adams, J.M., Cory, S., 2002. Apoptosomes: engines for caspase activation. Curr. Opin. Cell Biol. 14, 715–720.

Akao, M., Ohler, A., O'Rourke, B., Marban, E., 2001. Mitochondrial ATP-sensitive potassium channels inhibit apoptosis induced by oxidative stress in cardiac cells. Circ. Res. 88, 1267–1275.

Albano, J., Bhoola, K.D., Kingsley, G., 1977. The control of cyclic GMP by calcium, ionophore A23187, potassium and acetycholine in enzyme-secreting pancreatic slices. J. Physiol. (Lond.) 267, 35P–36P.

Allbritton, N.L., Verret, C.R., Wolley, R.C., Eisen, H.N., 1988. Calcium ion concentrations and DNA fragmentation in target cell destruction by murine cloned cytotoxic T lymphocytes. J. Exp. Med. 167, 514–527.

Anderson, P., 1997. Kinase cascades regulating entry into apoptosis. Microbiol. Mol. Biol. Rev. 61, 33–46.

Ankarcrona, M., Dypbukt, J.M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S.A., Nicotera, P., 1995. Glutamate-induced neuronal death: a succession of necrosis or apoptosis depending on mitochondrial function. Neuron 15, 961–973.

Antonsson, B., Conti, F., Ciavatta, A., Montessuit, S., Lewis, S., Martinou, I., Bernasconi, L., Bernard, A., Mermod, J.J., Mazzei, G., Maundrell, K., Gambale, F., Sadoul, R., Martinou, J.C., 1997. Inhibition of Bax channel-forming activity by Bcl-2. Science 277, 370–372.

Anwyl, R., 1977. Permeability of the post-synaptic membrane of an excitatory glutamate synapse to sodium and potassium. J. Physiol. (Lond.) 273, 367–388.

Archibald, J.T., White, T.D., 1974. Rapid reversal of internal Na^+ and K^+ contents of synaptosomes by ouabain. Nature 252, 595–596.

Ascher, P., Nowak, L., 1988. The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurones in culture. J. Physiol. (Lond.) 399, 247–266.

Ascher, P., Bregestovski, P., Nowak, L., 1988. *N*-Methyl-D-aspartate-activated channels of mouse central neurones in magnesium-free solutions. J. Physiol. (Lond.) 399, 207–226.

Avdonin, V., Kasuya, J., Ciorba, M.A., Kaplan, B., Hoshi, T., Iverson, L., 1998. Apoptotic proteins Reaper and Grim induce stable inactivation in voltage-gated K^+ channels. Proc. Natl. Acad. Sci. U.S.A. 95, 11703–11708.

Baczko, I., Lepran, I., Papp, J.G., 1997. KATP channel modulators increase survival rate during coronary occlusion–reperfusion in anaesthetized rats. Eur. J. Pharmacol. 324, 77–83.

Bajgar, R., Seetharaman, S., Kowaltowski, A.J., Garlid, K.D., Paucek, P., 2001. Identification and properties of a novel intracellular (mitochondrial) ATP-sensitive potassium channel in brain. J. Biol. Chem. 276, 33369–33374.

Balakirev, M.Y., Zimmer, G., 1998. Gradual changes in permeability of inner mitochondrial membrane precede the mitochondrial permeability transition. Arch. Biochem. Biophys. 356, 46–54.

Barbiero, G., Duranti, F., Bonelli, G., Amenta, J.S., Baccino, F.M., 1995. Intracellular ionic variations in the apoptotic death of L cells by inhibitors of cell cycle progression. Exp. Cell Res. 217, 410–418.

Beauvais, F., Michel, L., Dubertret, L., 1995. Human eosinophils in culture undergo a striking and rapid shrinkage during apoptosis. Role of K^+ channels. J. Leukoc. Biol. 57, 851–855.

Bennett, M.R., White, W., 1979. The survival and development of cholinergic neurons in potassium-enriched media. Brain Res. 173, 549–553.

Benson, R.S., Heer, S., Dive, C., Watson, A.J., 1996. Characterization of cell volume loss in CEM-C7A cells during dexamethasone-induced apoptosis. Am. J. Physiol. 270, C1190–C1203.

Bernardi, P., 1999. Mitochondrial transport of cations: channels, exchangers, and permeability transition. Physiol. Rev. 79, 1127–1155.

Bernardi, P., Vassanelli, S., Veronese, P., Colonna, R., Szabo, I., Zoratti, M., 1992. Modulation of the mitochondrial permeability transition pore. Effect of protons and divalent cations. J. Biol. Chem. 267, 2934–2939.

Bilney, A.J., Murray, A.W., 1998. Pro- and anti-apoptotic effects of K^+ in HeLa cells. FEBS Lett. 424, 221–224.

Blight, A.R., Toombs, J.P., Bauer, M.S., Widmer, W.R., 1991. The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: a phase I clinical trial. J. Neurotrauma 8, 103–119.

Bock, J., Szabo, I., Jekle, A., Gulbins, E., 2002. Actinomycin D-induced apoptosis involves the potassium channel Kv1.3. Biochem. Biophys. Res. Commun. 295, 526–531.

Bortner, C.D., Cidlowski, J.A., 1998. A necessary role for cell shrinkage in apoptosis. Biochem. Pharmacol. 56, 1549–1559.

Bortner, C.D., Cidlowski, J.A., 1999. Caspase independent/dependent regulation of K^+, cell shrinkage, and mitochondrial membrane potential during lymphocyte apoptosis. J. Biol. Chem. 274, 21953–21962.

Bortner, C.D., Hughes Jr., F.M., Cidlowski, J.A., 1997. A primary role for K^+ and Na^+ efflux in the activation of apoptosis. J. Biol. Chem. 272, 32436–32442.

Bortner, C.D., Gomez-Angelats, M., Cidlowski, J.A., 2001. Plasma membrane depolarization without repolarization is an early molecular event in anti-Fas-induced apoptosis. J. Biol. Chem. 276, 4304–4314.

Borzak, S., Al-Khaled, N., Douthat, L., Steinberg, M., Goldberg, A.D., 1997. Intravenous clofilium for conversion of atrial fibrillation. Am. J. Cardiol. 79, 94–97.

Budd, S.L., Lipton, S.A., 1999. Signaling events in NMDA receptor-induced apoptosis in cerebrocortical cultures. Ann. N. Y. Acad. Sci. 893, 261–264.

Buhrle, C.P., Sonnhof, U., 1983. The ionic mechanism of the excitatory action of glutamate upon the membranes of motoneurones of the frog. Pflugers Arch. 396, 154–162.

Burnashev, N., Schoepfer, R., Monyer, H., Ruppersberg, J.P., Gunther, W., Seeburg, P.H., Sakmann, B., 1992. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. Science 257, 1415–1419.

Cahn, F., Lubin, M., 1978. Inhibition of elongation steps of protein synthesis at reduced potassium concentrations in reticulocytes and reticulocyte lysate. J. Biol. Chem. 253, 7798–7803.

Chang, H., Ciani, S., Kidokoro, Y., 1994. Ion permeation properties of the glutamate receptor channel in cultured embryonic *Drosophila* myotubes. J. Physiol. (Lond.) 476, 1–16.

Chen, G.Q., Cui, C., Mayer, M.L., Gouaux, E., 1999. Functional characterization of a potassium-selective prokaryotic glutamate receptor. Nature 402, 817–821.

Chien, M.M., Zahradka, K.E., Newell, M.K., Freed, J.H., 1999. Fas-induced B cell apoptosis requires an increase in free cytosolic magnesium as an early event. J. Biol. Chem. 274, 7059–7066.

Chin, L.S., Park, C.C., Zitnay, K.M., Sinha, M., DiPatri Jr., A.J., Perillan, P., Simard, J.M., 1997. 4-Aminopyridine causes apoptosis and blocks an outward rectifier K^+ channel in malignant astrocytoma cell lines. J. Neurosci. Res. 48, 122–127.

Chiu, S.Y., Wilson, G.F., 1989. The role of potassium channels in Schwann cell proliferation in Wallerian degeneration of explant rabbit sciatic nerves. J. Physiol. (Lond.) 408, 199–222.

Choi, D.W., 1988a. Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. Trends Neurosci. 11, 465–469.

Choi, D.W., 1988b. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1, 623–634.

Choi, D.W., 1992. Excitotoxic cell death. J. Neurobiol. 23, 1261–1276.

Choi, D.W., Yu, S.P., Wei, L., 1998. Potassium channel blockers attenuate neuronal deaths induced by hypoxic insults in cortical culture and by transient focal ischemia in the rat. Soc. Neurosci. Abstr. 24, 1226.

Choi, B.Y., Kim, H.Y., Lee, K.H., Cho, Y.H., Kong, G., 1999. Clofilium, a potassium channel blocker, induces apoptosis of human promyelocytic leukemia (HL-60) cells via Bcl-2-insensitive activation of caspase-3. Cancer Lett. 147, 85–93.

Chvatal, A., Anderova, M., Ziak, D., Sykova, E., 1999. Glial depolarization evokes a larger potassium accumulation around oligodendrocytes than around astrocytes in gray matter of rat spinal cord slices. J. Neurosci. Res. 56, 493–505.

Clausen, T., 1996. The Na^+, K^+ pump in skeletal muscle: quantification, regulation and functional significance. Acta Physiol. Scand. 156, 227–235.

Collins, F., Schmidt, M.F., Guthrie, P.B., Kater, S.B., 1991. Sustained increase in intracellular calcium promotes neuronal survival. J. Neurosci. 11, 2582–2587.

Colom, L.V., Diaz, M.E., Beers, D.R., Neely, A., Xie, W.J., Appel, S.H., 1998. Role of potassium channels in amyloid-induced cell death. J. Neurochem. 70, 1925–1934.

Coma, M., Vicente, R., Busquets, S., Carbo, N., Tamkun, M.M., Lopez-Soriano, F.J., Argiles, J.M., Felipe, A., 2003. Impaired voltage-gated K^+ channel expression in brain during experimental cancer cachexia. FEBS Lett. 536, 45–50.

Cone Jr., C.D., Cone, C.M., 1976. Induction of mitosis in mature neurons in central nervous system by sustained depolarization. Science 192, 155–158.

Cornelius, F., Mahmmoud, Y.A., Christensen, H.R., 2001. Modulation of Na, K-ATPase by associated small transmembrane regulatory proteins and by lipids. J. Bioenerg. Biomembr. 33, 415–423.

Cowan, A.I., Martin, R.L., 1992. Ionic basis of membrane potential changes induced by anoxia in rat dorsal vagal motoneurones. J. Physiol. (Lond.) 455, 89–109.

Crompton, M., 1999. The mitochondrial permeability transition pore and its role in cell death. Biochem. J. 341, 233–249.

Dallaporta, B., Hirsch, T., Susin, S.A., Zamzami, N., Larochette, N., Brenner, C., Marzo, I., Kroemer, G., 1998. Potassium leakage during the apoptotic degradation phase. J. Immunol. 160, 5605–5615.

Dallaporta, B., Marchetti, P., de Pablo, M.A., Maisse, C., Duc, H.T., Metivier, D., Zamzami, N., Geuskens, M., Kroemer, G., 1999. Plasma membrane potential in thymocyte apoptosis. J. Immunol. 162, 6534–6542.

Daniels, M., Brown, D.R., 2002. High extracellular potassium protects against the toxicity of cytosine arabinoside but is not required for the survival of cerebellar granule cells in vitro. Mol. Cell. Neurosci. 19, 281–291.

Deckers, C.L., Lyons, A.B., Samuel, K., Sanderson, A., Maddy, A.H., 1993. Alternative pathways of apoptosis induced by methylprednisolone and valinomycin analyzed by flow cytometry. Exp. Cell Res. 208, 362–370.

DeCoursey, T.E., Chandy, K.G., Gupta, S., Cahalan, M.D., 1984. Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? Nature 307, 465–468.

Dekin, M.S., 1983. Permeability changes induced by L-glutamate at the crayfish neuromuscular junction. J. Physiol. (Lond.) 341, 105–125.

de Luca, A., Weller, M., Fontana, A., 1996. TGF-beta-induced apoptosis of cerebellar granule neurons is prevented by depolarization. J. Neurosci. 16, 4174–4185.

Diem, R., Meyer, R., Weishaupt, J.H., Bahr, M., 2001. Reduction of potassium currents and phosphatidylinositol 3-kinase-dependent AKT phosphorylation by tumor necrosis factor-α rescues axotomized retinal ganglion cells from retrograde cell death in vivo. J. Neurosci. 21, 2058–2066.

DiPolo, R., Beauge, L., 1991. Regulation of Na–Ca exchange. An overview. Ann. N. Y. Acad. Sci. 639, 100–111.

Diwan, J.J., Haley, T., Sanadi, D.R., 1988. Reconstitution of transmembrane K^+ transport with a 53 kilodalton mitochondrial protein. Biochem. Biophys. Res. Commun. 153, 224–230.

Dobretsov, M., Stimers, J.R., 1996. Characterization of the Na/K pump current in N20.1 oligodendrocytes. Brain Res. 724, 103–111.

Dos Santos, P., Kowaltowski, A.J., Laclau, M.N., Seetharaman, S., Paucek, P., Boudina, S., Thambo, J.B., Tariosse, L., Garlid, K.D., 2002. Mechanisms by which opening the mitochondrial ATP- sensitive K^+ channel protects the ischemic heart. Am. J. Physiol. Heart Circ. Physiol. 283, H284–H295.

Du, C., Hu, R., Csernansky, C.A., Hsu, C.Y., Choi, D.W., 1996. Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J. Cereb. Blood Flow Metab. 16, 195–201.

Duff, H.J., Mitchell, L.B., Wyse, D.G., Gillis, A.M., Sheldon, R.S., 1991. Mexiletine/quinidine combination therapy: electrophysiologic correlates of anti-arrhythmic efficacy. Clin. Invest. Med. 14, 476–483.

Duggan, P.F., 1977. An enzyme system requiring magnesium, calcium and potassium ions. Proc. R. Ir. Acad. B 77, 449–455.

Duke, R.C., Witter, R.Z., Nash, P.B., Young, J.D., Ojcius, D.M., 1994. Cytolysis mediated by ionophores and pore-forming agents: role of intracellular calcium in apoptosis. FASEB J. 8, 237–246.

Eckstein-Ludwig, U., Rettinger, J., Vasilets, L.A., Schwarz, W., 1998. Voltage-dependent inhibition of the Na^+, K^+ pump by tetraethylammonium. Biochim. Biophys. Acta 1372, 289–300.

Edwards, G., Weston, A.H., 1995. Pharmacology of the potassium channel openers. Cardiovasc. Drugs Ther. 9 (Suppl. 2), 185–193.

Eggleston, T.L.V., Krebs, H., 1950. The role of glutamic acid in the transport of potassium in brain and retina. J. Physiol. (Lond.) 47, 139–149.

Eliseev, R.A., Salter, J.D., Gunter, K.K., Gunter, T.E., 2003. Bcl-2 and tBid proteins counter-regulate mitochondrial potassium transport. Biochim. Biophys. Acta 1604, 1–5.

Elliott, J.I., Higgins, C.F., 2003. IKCa1 activity is required for cell shrinkage, phosphatidylserine translocation and death in T lymphocyte apoptosis. EMBO Rep. 4, 189–194.

Ekhterae, D., Platoshyn, O., Krick, S., Yu, Y., McDaniel, S.S., Yuan, J.X., 2001. Bcl-2 decreases voltage-gated K^+ channel activity and enhances survival in vascular smooth muscle cells. Am. J. Physiol. Cell Physiol. 281, C157–C165.

Enokido, Y., Hatanaka, H., 1993. Apoptotic cell death occurs in hippocampal neurons cultured in a high oxygen atmosphere. Neuroscience 57, 965–972.

Evan, G., Harrington, E., Fanidi, A., Land, H., Amati, B., Bennett, M., 1994. Integrated control of cell proliferation and cell death by the c-*myc* oncogene. Philos. Trans. R. Soc. Lond. B Biol. Sci. 345, 269–275.

Fan, Z., Neff, R.A., 2000. Susceptibility of ATP-sensitive K^+ channels to cell stress through mediation of phosphoinositides as examined by photoirradiation. J. Physiol. (Lond.) 529, 707–721.

Farkas, D.L., Wei, M.D., Febbroriello, P., Carson, J.H., Loew, L.M., 1989. Simultaneous imaging of cell and mitochondrial membrane potentials. Biophys. J. 56, 1053–1069.

Feldenberg, L.R., Thevananther, S., del Rio, M., de Leon, M., Devarajan, P., 1999. Partial ATP depletion induces Fas- and caspase-mediated apoptosis in MDCK cells. Am. J. Physiol. 276, F837–F846.

Fernandez-Segura, E., Canizares, F.J., Cubero, M.A., Warley, A., Campos, A., 1999. Changes in elemental content during apoptotic cell death studied by electron probe X-ray microanalysis. Exp. Cell Res. 253, 454–462.

Ferrari, G., Greene, L.A., 1994. Proliferative inhibition by dominant-negative Ras rescues naive and neuronally differentiated PC12 cells from apoptotic death. EMBO J. 13, 5922–5928.

Fischer, E.H., 1999. Cell signaling by protein tyrosine phosphorylation. Adv. Enzyme Regul. 39, 359–369.

Forbes,
R.A., Steenbergen, C., Murphy, E., 2001. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. Circ. Res. 88, 802–809.

Fordyce, C.B., Jagasia, R., Schlichter, J.C., 2002. Modulation of microglia-induced neurotoxicity (poster title in the meeting: K^+ channel blockers reduce microglia-induced neurotoxicity). Soc. Neurosci. Abstr. 17, 892.

Franklin, J.L., Johnson Jr., E.M., 1992. Suppression of programmed neuronal death by sustained elevation of cytoplasmic calcium. Trends Neurosci. 15, 501–508.

Fujita, H., Takizawa, S., Nanri, K., Matsushima, K., Ogawa, S., Shinohara, Y., 1997. Potassium channel opener reduces extracellular glutamate concentration in rat focal cerebral ischemia. Brain Res. Bull. 43, 365–368.

Furlong, I.J., Lopez Mediavilla, C., Ascaso, R., Lopez Rivas, A., Collins, M.K., 1998. Induction of apoptosis by valinomycin: mitochondrial permeability transition causes intracellular acidification. Cell Death Differ. 5, 214–221.

Furukawa, K., Barger, S.W., Blalock, E.M., Mattson, M.P., 1996. Activation of K^+ channels and suppression of neuronal activity by secreted beta-amyloid-precursor protein. Nature 379, 74–78.

Gallagher, J.D., Fay, M.J., North, W.G., McCann, F.V., 1996. Ionic signals in T47D human breast cancer cells. Cell Signal. 8, 279–284.

Gallo, V., Kingsbury, A., Balazs, R., Jorgensen, O.S., 1987. The role of depolarization in the survival and differentiation of cerebellar granule cells in culture. J. Neurosci. 7, 2203–2213.

Gantner, F., Uhlig, S., Wendel, A., 1995. Quinine inhibits release of tumor necrosis factor, apoptosis, necrosis and mortality in a murine model of septic liver failure. Eur. J. Pharmacol. 294, 353–355.

Garlid, K.D., Paucek, P., 2001. The mitochondrial potassium cycle. IUBMB Life 52, 153–158.

Garlid, K.D., Paucek, P., Yarov-Yarovoy, V., Sun, X., Schindler, P.A., 1996. The mitochondrial KATP channel as a receptor for potassium channel openers. J. Biol. Chem. 271, 8796–8799.

Gary, D.S., Mattson, M.P., 2001. Integrin signaling via the PI3-kinase-Akt pathway increases neuronal resistance to glutamate-induced apoptosis. J. Neurochem. 76, 1485–1496.

Ghosh, S., Standen, N.B., Galinanes, M., 2000. Evidence for mitochondrial K ATP channels as effectors of human myocardial preconditioning. Cardiovasc. Res. 45, 934–940.

Gloor, S.M., 1997. Relevance of Na, K-ATPase to local extracellular potassium homeostasis and modulation of synaptic transmission. FEBS Lett. 412, 1–4.

Goldberg, M.P., 2002. Stroke Trials Directory. Internet Stroke Center at Washington University (http://www.stroketrials.org). Web page for BMS-204352: http://www.strokecenter.org/trials/InterventionDetail.asp?therapyName=ints/intPage53.htm, accessed on 24 April 2002.

Gomez-Angelats, M., Bortner, C.D., Cidlowski, J.A., 2000. Protein kinase C (PKC) inhibits fas receptor-induced apoptosis through modulation of the loss of K^+ and cell shrinkage. A role for PKC upstream of caspases. J. Biol. Chem. 275, 19609–19619.

Goodman, Y., Mattson, M.P., 1996. K^+ channel openers protect hippocampal neurons against oxidative injury and amyloid beta-peptide toxicity. Brain Res. 706, 328–332.

Greene, H.L., Graham, E.L., Werner, J.A., Sears, G.K., Gross, B.W., Gorham, J.P., Kudenchuk, P.J., Trobaugh, G.B., 1983. Toxic and therapeutic effects of amiodarone in the treatment of cardiac arrhythmias. J. Am. Coll. Cardiol. 2, 1114–1128.

Gribkoff, V.K., Starrett Jr., J.E., Dworetzky, S.I., Hewawasam, P., Boissard, C.G., Cook, D.A., Frantz, S.W., Heman, K., Hibbard, J.R., Huston, K., Johnson, G., Krishnan, B.S., Kinney, G.G., Lombardo, L.A., Meanwell, N.A., Molinoff, P.B., Myers, R.A., Moon, S.L., Ortiz, A., Pajor, L., Pieschl, R.L., Post-Munson, D.J., Signor, L.J., Srinivas, N., Taber, M.T., Thalody, G., Trojnacki, J.T., Wiener, H., Yeleswaram, K., Yeola, S.W., 2001. Targeting acute ischemic stroke with a calcium-sensitive opener of maxi-K potassium channels. Nat. Med. 7, 471–477.

Gross, G.J., Fryer, R.M., 1999. Sarcolemmal versus mitochondrial ATP-sensitive K^+ channels and myocardial preconditioning. Circ. Res. 84, 973–979.

Grover, G.J., 1997. Pharmacology of ATP-sensitive potassium channel (KATP) openers in models of myocardial ischemia and reperfusion. Can J. Physiol. Pharmacol. 75, 309–315.

Grover, G.J., Garlid, K.D., 2000. ATP-sensitive potassium channels: a review of their cardioprotective pharmacology. J. Mol. Cell. Cardiol. 32, 677–695.

Guatteo, E., Federici, M., Siniscalchi, A., Knopfel, T., Mercuri, N.B., Bernardi, G., 1998. Whole cell patch-clamp recordings of rat midbrain dopaminergic neurons isolate a sulphonylurea- and ATP-sensitive component of potassium currents activated by hypoxia. J. Neurophysiol. 79, 1239–1245.

Guizouarn, H., Motais, R., Garcia-Romeu, F., Borgese, F., 2000. Cell volume regulation: the role of taurine loss in maintaining membrane potential and cell pH. J. Physiol. (Lond.) 523, 147–154.

Haddad, G.G., 1997. K^+ ions and channels: mechanisms of sensing O_2 deprivation in central neurons. Kidney Int. 51, 467–472.

Halter, J.A., Blight, A.R., Donovan, W.H., Calvillo, O., 2000. Intrathecal administration of 4-aminopyridine in chronic spinal injured patients. Spinal Cord 38, 728–732.

Hansebout, R.R., Blight, A.R., Fawcett, S., Reddy, K., 1993. 4-Amino-pyridine in chronic spinal cord injury: a controlled, double-blind, crossover study in eight patients. J. Neurotrauma 10, 1–18.

Harvey, A.M.H., 1968. Excitatory acidic amino acids and the cation content and sodium ion flux of isolated tissues from the brain. Biochem. J. 108, 269–274.

Hayes, K.C., Blight, A.R., Potter, P.J., Allatt, R.D., Hsieh, J.T., Wolfe, D.L., Lam, S., Hamilton, J.T., 1993. Preclinical trial of 4-aminopyridine in patients with chronic spinal cord injury. Paraplegia 31, 216–224.

Hengartner, M.O., Ellis, R.E., Horvitz, H.R., 1992. *Caenorhabditis elegans* gene *ced-9* protects cells from programmed cell death. Nature 356, 494–499.

Hertz, L., 1992. Autonomic control of neuronal–astrocytic interactions, regulating metabolic activities, and ion fluxes in the CNS. Brain Res. Bull. 29, 303–313.

Hida, H., Takeda, M., Soliven, B., 1998. Ceramide inhibits inwardly rectifying K^+ currents via a Ras- and Raf-1-dependent pathway in cultured oligodendrocytes. J. Neurosci. 18, 8712–8719.

Hirsch, T., Marchetti, P., Susin, S.A., Dallaporta, B., Zamzami, N., Marzo, I., Geuskens, M., Kroemer, G., 1997. The apoptosis–necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. Oncogene 15, 1573–1581.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Holmes, T.C., Berman, K., Swartz, J.E., Dagan, D., Levitan, I.B., 1997. Expression of voltage-gated potassium channels decreases cellular protein tyrosine phosphorylation. J. Neurosci. 17, 8964–8974.

Houzen, H., Kikuchi, S., Kanno, M., Shinpo, K., Tashiro, K., 1997. Tumor necrosis factor enhancement of transient outward potassium currents in cultured rat cortical neurons. J. Neurosci. Res. 50, 990–999.

Huang, H., Gao, T.M., Gong, L., Zhuang, Z., Li, X., 2001. Potassium channel blocker TEA prevents CA1 hippocampal injury following transient forebrain ischemia in adult rats. Neurosci. Lett. 305, 83–86.

Hughes Jr., F.M., Cidlowski, J.A., 1999. Potassium is a critical regulator of apoptotic enzymes in vitro and in vivo. Adv. Enzyme Regul. 39, 157–171.

Hughes Jr., F.M., Bortner, C.D., Purdy, G.D., Cidlowski, J.A., 1997. Intracellular K^+ suppresses the activation of apoptosis in lymphocytes. J. Biol. Chem. 272, 30567–30576.

Ichinose, T., Yu, S.P., 2000. Unique regulation of the K^+ current mediated by NMDA receptor channel in mouse cortical neurons. Soc. Neurosci. Abstr. 26, 2147.

Ichinose, M., Yonemochi, H., Sato, T., Saikawa, T., 2003a. Diazoxide triggers cardioprotection against apoptosis induced by oxidative stress. Am. J. Physiol. Heart Circ. Physiol. 284, H2235–H2241.

Ichinose T., Yu S., Wang X.Q., Yu S.P., 2003b. Ca^{2+}-independent but voltage/activity-dependent regulation of NMDA receptor-mediated outward K^+ current in cortical neurons. J. Physiol., in press.

Iino, M., Ozawa, S., Tsuzuki, K., 1990. Permeation of calcium through excitatory amino acid receptor channels in cultured rat hippocampal neurones. J. Physiol. (Lond.) 424, 151–165.

Inai, Y., Yabuki, M., Kanno, T., Akiyama, J., Yasuda, T., Utsumi, K., 1997. Valinomycin induces apoptosis of ascites hepatoma cells (AH-130) in relation to mitochondrial membrane potential. Cell Struct. Funct. 22, 555–563.

Inoue, I., Nagase, H., Kishi, K., Higuti, T., 1991. ATP-sensitive K^+ channel in the mitochondrial inner membrane. Nature 352, 244–247.

Ishaque, A., Al-Rubeai, M., 1999. Role of Ca^{2+}, Mg^{2+} and K^+ ions in determining apoptosis and extent of suppression afforded by bcl-2 during hybridoma cell culture. Apoptosis 4, 335–355.

Itoh, T., Seki, N., Suzuki, S., Ito, S., Kajikuri, J., Kuriyama, H., 1992. Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. J. Physiol. (Lond.) 451, 307–328.

Jacobson, M.D., Evan, G.I., 1994. Apoptosis. Breaking the ICE. Curr. Biol. 4, 337–340.

Jacotot, E., Costantini, P., Laboureau, E., Zamzami, N., Susin, S.A., Kroemer, G., 1999. Mitochondrial membrane permeabilization during the apoptotic process. Ann. N. Y. Acad. Sci. 887, 18–30.

Jalonen, T.O., Charniga, C.J., Wielt, D.B., 1997. β-Amyloid peptide-induced morphological changes coincide with increased K^+ and Cl^- channel activity in rat cortical astrocytes. Brain Res. 746, 85–97.

Jan, L.Y., Jan, Y.N., 1976. L-Glutamate as an excitatory transmitter at the *Drosophila* larval neuromuscular junction. J. Physiol. (Lond.) 262, 215–236.

Jan, L.Y., Jan, Y.N., 1997. Cloned potassium channels from eukaryotes and prokaryotes. Annu. Rev. Neurosci. 20, 91–123.

Jiang, C., Haddad, G.G., 1993. Short periods of hypoxia activate a K^+ current in central neurons. Brain Res. 614, 352–356.

Johnson Jr., E.M., Koike, T., Franklin, J., 1992. A "calcium set-point hypothesis" of neuronal dependence on neurotrophic factor. Exp. Neurol. 115, 163–166.

Johnson Jr., E.M., Greenlund, L.J., Akins, P.T., Hsu, C.Y., 1995. Neuronal apoptosis: current understanding of molecular mechanisms and potential role in ischemic brain injury. J. Neurotrauma 12, 843–852.

Johnston, A.R., Fraser, J.R., Jeffrey, M., MacLeod, N., 1998. Alterations in potassium currents may trigger neurodegeneration in murine scrapie. Exp. Neurol. 151, 326–333.

Jonas, D., Walev, I., Berger, T., Liebetrau, M., Palmer, M., Bhakdi, S., 1994. Novel path to apoptosis: small transmembrane pores created by staphylococcal alpha-toxin in T lymphocytes evoke internucleosomal DNA degradation. Infect. Immun. 62, 1304–1312.

Jones, R.E., Heron, J.R., Foster, D.H., Snelgar, R.S., Mason, R.J., 1983. Effects of 4-aminopyridine in patients with multiple sclerosis. J. Neurol. Sci. 60, 353–362.

Jurkowitz-Alexander, M.S., Altschuld, R.A., Hohl, C.M., Johnson, J.D., McDonald, J.S., Simmons, T.D., Horrocks, L.A., 1992. Cell swelling, blebbing, and death are dependent on ATP depletion and independent of calcium during chemical hypoxia in a glial cell line (ROC-1). J. Neurochem. 59, 344–352.

Kawasaki, H., Morooka, T., Shimohama, S., Kimura, J., Hirano, T., Gotoh, Y., Nishida, E., 1997. Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J. Biol. Chem. 272, 18518–18521.

Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26, 239–257.

Kim, Y.H., Kim, E.Y., Gwag, B.J., Sohn, S., Koh, J.Y., 1999. Zinc-induced cortical neuronal death with features of apoptosis and necrosis: mediation by free radicals. Neuroscience 89, 175–182.

Kim, J.A., Kang, Y.S., Jung, M.W., Kang, G.H., Lee, S.H., Lee, Y.S., 2000. Ca^{2+} influx mediates apoptosis induced by 4-aminopyridine, a K^+ channel blocker, in HepG2 human hepatoblastoma cells. Pharmacology 60, 74–81.

Kitamura, K., Kamouchi, M., 1993. K channel openers activate different K channels in vascular smooth muscle cells. Cardiovasc. Drugs Ther. 7 (Suppl. 3), 539–546.

Knutson, P., Ghiani, C.A., Zhou, J.M., Gallo, V., McBain, C.J., 1997. K^+ channel expression and cell proliferation are regulated by intracellular sodium and membrane depolarization in oligodendrocyte progenitor cells. J. Neurosci. 17, 2669–2682.

Koh, J.Y., Gwag, B.J., Lobner, D., Choi, D.W., 1995. Potentiated necrosis of cultured cortical neurons by neurotrophins. Science 268, 573–575.

Koike, T., Martin, D.P., Johnson Jr., E.M., 1989. Role of Ca^{2+} channels in the ability of membrane depolarization to prevent neuronal death induced by trophic-factor deprivation: evidence that levels of internal Ca^{2+} determine nerve growth factor dependence of sympathetic ganglion cells. Proc. Natl. Acad. Sci. U.S.A. 86, 6421–6425.

Komatsu, N., Nakagawa, M., Oda, T., Muramatsu, T., 2000. Depletion of intracellular NAD^+ and ATP levels during ricin-induced apoptosis through the specific ribosomal inactivation results in the cytolysis of U937 cells. J. Biochem. (Tokyo) 128, 463–470.

Kopia, G.A., Eller, B.T., Patterson, E., Shea M, J., Lucchesi, B.R., 1985. Antiarrhythmic and electrophysiologic actions of clofilium in experimental canine models. Eur. J. Pharmacol. 116, 49–61.

Kovac, L., Bohmerova, E., Butko, P., 1982. Ionophores and intact cells. I. Valinomycin and nigericin act preferentially on mitochondria and not on the plasma membrane of *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 721, 341–348.

Kowaltowski, A.J., Seetharaman, S., Paucek, P., Garlid, K.D., 2001. Bioenergetic consequences of opening the ATP-sensitive K^+ channel of heart mitochondria. Am. J. Physiol. Heart Circ. Physiol. 280, H649–H657.

Krick, S., Platoshyn, O., Sweeney, M., Kim, H., Yuan, J.X., 2001. Activation of K^+ channels induces apoptosis in vascular smooth muscle cells. Am. J. Physiol. Cell Physiol. 280, C970–C979.

Krick, S., Platoshyn, O., Sweeney, M., McDaniel, S.S., Zhang, S., Rubin, L.J., Yuan, J.X., 2002. Nitric oxide induces apoptosis by activating K^+ channels in pulmonary vascular smooth muscle cells. Am. J. Physiol. Heart Circ. Physiol. 282, H184–H193.

Kroemer, G., Reed, J.C., 2000. Mitochondrial control of cell death. Nat. Med. 6, 513–519.

Kroemer, G., Petit, P., Zamzami, N., Vayssiere, J.L., Mignotte, B., 1995. The biochemistry of programmed cell death. FASEB J. 9, 1277–1287.

Kumar, A.R., Kurup, P.A., 2002. Inhibition of membrane Na^+–K^+ ATPase activity: a common pathway in central nervous system disorders. J. Assoc. Phys. India 50, 400–406.

Latta, M., Kunstle, G., Leist, M., Wendel, A., 2000. Metabolic depletion of ATP by fructose inversely controls CD95- and tumor necrosis factor receptor 1-mediated hepatic apoptosis. J. Exp. Med. 191, 1975–1985.

Lauritzen, I., De Weille, J.R., Lazdunski, M., 1997. The potassium channel opener (−)-cromakalim prevents glutamate-induced cell death in hippocampal neurons. J. Neurochem. 69, 1570–1579.

Lavretsky, E.P., Jarvik, L.F., 1992. A group of potassium-channel blockers-acetylcholine releasers: new potentials for Alzheimer disease? A review. J. Clin. Psychopharmacol. 12, 110–118.

Ledbetter, M.L., Lubin, M., 1977. Control of protein synthesis in human fibroblasts by intracellular potassium. Exp. Cell Res. 105, 223–236.

Lesort, M., Esclaire, F., Yardin, C., Hugon, J., 1997. NMDA induces apoptosis and necrosis in neuronal cultures. Increased APP immunoreactivity is linked to apoptotic cells. Neurosci. Lett. 221, 213–216.

Li, H., Yuan, J., 1999. Deciphering the pathways of life and death. Curr. Opin. Cell Biol. 11, 261–266.

Lijnen, P., Hespel, P., Lommelen, G., Laermans, M., M'Buyamba-Kabangu, J.R., Amery, A., 1986. Intracellular sodium, potassium and magnesium concentration, ouabain-sensitive ^{86}rubidium-uptake and sodium-efflux and Na^+, K^+-cotransport activity in erythrocytes of normal male subjects studied on two occasions. Methods Find. Exp. Clin. Pharmacol. 8, 525–533.

Liu, Y., Sato, T., O'Rourke, B., Marban, E., 1998. Mitochondrial ATP-dependent potassium channels: novel effectors of cardioprotection? Circulation 97, 2463–2469.

Liu, Y., Sato, T., Seharaseyon, J., Szewczyk, A., O'Rourke, B., Marban, E., 1999. Mitochondrial ATP-dependent potassium channels. Viable candidate effectors of ischemic preconditioning. Ann. N. Y. Acad. Sci. 874, 27–37.

Lopina, O.D., 2001. Interaction of Na, K-ATPase catalytic subunit with cellular proteins and other endogenous regulators. Biochemistry (Mosc.) 66, 1122–1131.

Lubin, M., 1967. Intracellular potassium and macromolecular synthesis in mammalian cells. Nature 213, 451–453.

MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J., Barker, J.L., 1986. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature 321, 519–522.

Maeno, E., Ishizaki, Y., Kanaseki, T., Hazama, A., Okada, Y., 2000. Normotonic cell shrinkage because of disordered volume regulation is an early prerequisite to apoptosis. Proc. Natl. Acad. Sci. U.S.A. 97, 9487–9492.

Majno, G., Joris, I., 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. Am. J. Pathol. 146, 3–15.

Malin, S.A., Guo, W.X., Jafari, G., Goate, A.M., Nerbonne, J.M., 1998. Presenilins upregulate functional K^+ channel currents in mammalian cells. Neurobiol. Dis. 4, 398–409.

Manikkam, M., Li, Y., Mitchell, B.M., Mason, D.E., Freeman, L.C., 2002. Potassium channel antagonists influence porcine granulosa cell proliferation, differentiation, and apoptosis. Biol. Reprod. 67, 88–98.

Mann, C.L., Bortner, C.D., Jewell, C.M., Cidlowski, J.A., 2001. Glucocorticoid-induced plasma membrane depolarization during thymocyte apoptosis: association with cell shrinkage and degradation of the Na^+/K^+-adenosine triphosphatase. Endocrinology 142, 5059–5068.

Marchetti, P., Susin, S.A., Decaudin, D., Gamen, S., Castedo, M., Hirsch, T., Zamzami, N., Naval, J., Senik, A., Kroemer, G., 1996. Apoptosis-associated derangement of mitochondrial function in cells lacking mitochondrial DNA. Cancer Res. 56, 2033–2038.

Marklund, L., Behnam-Motlagh, P., Henriksson, R., Grankvist, K., 2001a. Bumetanide annihilation of amphotericin B-induced apoptosis and cytotoxicity is due to its effect on cellular K^+ flux. J. Antimicrob. Chemother. 48, 781–786.

Marklund, L., Henriksson, R., Grankvist, K., 2001b. Cisplatin-induced apoptosis of mesothelioma cells is affected by potassium ion flux modulator amphotericin B and bumetanide. Int. J. Cancer 93, 577–583.

Matthews, G., Wickelgren, W.O., 1979. Glutamate and synaptic excitation of reticulospinal neurones of lamprey. J. Physiol. (Lond.) 293, 417–433.

Mayer, M.L., Westbrook, G.L., 1987a. The physiology of excitatory amino acids in the vertebrate central nervous system. Prog. Neurobiol. 28, 197–276.

Mayer, M.L., Westbrook, G.L., 1987b. Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. J. Physiol. (Lond.) 394, 501–527.

McCabe, R.D., Young, D.B., 1994. Potassium inhibits cultured vascular smooth muscle cell proliferation. Am. J. Hypertens. 7, 346–350.

McCarthy, J.V., Cotter, T.G., 1997. Cell shrinkage and apoptosis: a role for potassium and sodium ion efflux. Cell Death Differ. 4, 756–770.

McConkey, D.J., Lin, Y., Nutt, L.K., Ozel, H.Z., Newman, R.A., 2000. Cardiac glycosides stimulate Ca^{2+} increases and apoptosis in androgen-independent, metastatic human prostate adenocarcinoma cells. Cancer Res. 60, 3807–3812.

McLaughlin, B., Pal, S., Tran, M.P., Parsons, A.A., Barone, F.C., Erhardt, J.A., Aizenman, E., 2001. p38 activation is required upstream of potassium current enhancement and caspase cleavage in thiol oxidant-induced neuronal apoptosis. J. Neurosci. 21, 3303–3311.

Menasche, P., Kevelaitis, E., Mouas, C., Grousset, C., Piwnica, A., Bloch, G., 1995. Preconditioning with potassium channel openers. A new concept for enhancing cardioplegic protection? J. Thorac. Cardiovasc. Surg. 110, 1606–1613.

Minn, A.J., Velez, P., Schendel, S.L., Liang, H., Muchmore, S.W., Fesik, S.W., Fill, M., Thompson, C.B., 1997. Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature 385, 353–357.

Mironov, S.L., Richter, D.W., 2000. Hypoxic modulation of L-type Ca^{2+} channels in inspiratory brainstem neurones: intracellular signalling pathways and metabotropic glutamate receptors. Brain Res. 869, 166–177.

Mizumura, T., Nithipatikom, K., Gross, G.J., 1995. Bimakalim, an ATP-sensitive potassium channel opener, mimics the effects of ischemic preconditioning to reduce infarct size, adenosine release, and neutrophil function in dogs. Circulation 92, 1236–1245.

Monaghan, D.T., Bridges, R.J., Cotman, C.W., 1989. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 29, 365–402.

Montague, J.W., Bortner, C.D., Hughes Jr., F.M., Cidlowski, J.A., 1999. A necessary role for reduced intracellular potassium during the DNA degradation phase of apoptosis. Steroids 64, 563–569.

Muchmore, S.W., Sattler, M., Liang, H., Meadows, R.P., Harlan, J.E., Yoon, H.S., Nettesheim, D., Chang, B.S., Thompson, C.B., Wong, S.L., Ng, S.L., Fesik, S.W., 1996. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature 381, 335–341.

Murphy, K.P., Greenfield, S.A., 1991. ATP-sensitive potassium channels counteract anoxia in neurones of the substantia nigra. Exp. Brain Res. 84, 355–358.

Murray, N.M., Newsom-Davis, J., 1981. Treatment with oral 4-aminopyridine in disorders of neuromuscular transmission. Neurology 31, 265–271.

Nadeau, H., McKinney, S., Anderson, D.J., Lester, H.A., 2000. ROMK1 (Kir1.1) causes apoptosis and chronic silencing of hippocampal neurons. J. Neurophysiol. 84, 1062–1075.

Nagy, P., Panyi, G., Jenei, A., Bene, L., Gaspar Jr., R., Matko, J., Damjanovich, S., 1995. Ion-channel activities regulate transmembrane signaling in thymocyte apoptosis and T-cell activation. Immunol. Lett. 44, 91–95.

Nakajima, W., Ishida, A., Lange, M.S., Gabrielson, K.L., Wilson, M.A., Martin, L.J., Blue, M.E., Johnston, M.V., 2000. Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. J. Neurosci. 20, 7994–8004.

Niethammer, M., Kim, E., Sheng, M., 1996. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J. Neurosci. 16, 2157–2163.

Nietsch, H.H., Roe, M.W., Fiekers, J.F., Moore, A.L., Lidofsky, S.D., 2000. Activation of potassium and chloride channels by tumor necrosis factor alpha. Role in liver cell death. J. Biol. Chem. 275, 20556–20561.

Nilius, B., Wohlrab, W., 1992. Potassium channels and regulation of proliferation of human melanoma cells. J. Physiol. (Lond.) 445, 537–548.

Nishi, R., Berg, D.K., 1981. Effects of high K^+ concentrations on the growth and development of ciliary ganglion neurons in cell culture. Dev. Biol. 87, 301–307.

Nord, L.D., Stolfi, R.L., Alfieri, A.A., Netto, G., Reuter, V., Sternberg, S.S., Colofiore, J.R., Koutcher, J.A., Martin, D.S., 1997. Apoptosis induced in advanced CD8F1-murine mammary tumors by the combination of PALA, MMPR and 6AN precedes tumor regression and is preceded by ATP depletion. Cancer Chemother. Pharmacol. 40, 376–387.

Norman, D.J., Feng, L., Cheng, S.S., Gubbay, J., Chan, E., Heintz, N., 1995. The lurcher gene induces apoptotic death in cerebellar Purkinje cells. Development 121, 1183–1193.

Obata, T., Yamanaka, Y., 2000. Block of cardiac ATP-sensitive K^+ channels reduces hydroxyl radicals in the rat myocardium. Arch. Biochem. Biophys. 378, 195–200.

Ojcius, D.M., Zychlinsky, A., Zheng, L.M., Young, J.D., 1991. Ionophore-induced apoptosis: role of DNA fragmentation and calcium fluxes. Exp. Cell Res. 197, 43–49.

Okada, Y., Maeno, E., 2001. Apoptosis, cell volume regulation and volume-regulatory chloride channels. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 130, 377–383.

Olej, B., dos Santos, N.F., Leal, L., Rumjanek, V.M., 1998. Ouabain induces apoptosis on PHA-activated lymphocytes. Biosci. Rep. 18, 1–7.

Olney, J.W., 1990. Excitotoxicity: an overview. Can. Dis. Wkly. Rep. 16 (Suppl. 1E), 47–57.

O'Reilly, N., Xia, Z., Fiander, H., Tauskela, J., Small, D.L., 2002. Disparity between ionic mediators of volume regulation and apoptosis in N1E 115 mouse neuroblastoma cells. Brain Res. 943, 245–256.

Ozawa, S., Nakamura, T., Yuzaki, M., 1988. Cation permeability change caused by L-glutamate in cultured rat hippocampal neurons. Brain Res. 443, 85–94.

Pal, S.K., Du, S., Hartnett, K.A., Levitan, E.S., Aizenman, E., 2002. Involvement of the potassium channel subunit Kv2.1 in oxidative stress mediated cell death. Soc. Neurosci. Abstr. 12, 493.

Pappone, P.A., Ortiz-Miranda, S.I., 1993. Blockers of voltage-gated K channels inhibit proliferation of cultured brown fat cells. Am. J. Physiol. 264, C1014–C1019.

Patel, T., Bronk, S.F., Gores, G.J., 1994. Increases of intracellular magnesium promote glycodeoxycholate-induced apoptosis in rat hepatocytes. J. Clin. Invest. 94, 2183–2192.

Paucek, P., Mironova, G., Mahdi, F., Beavis, A.D., Woldegiorgis, G., Garlid, K.D., 1992. Reconstitution and partial purification of the glibenclamide-sensitive, ATP-dependent K^+ channel from rat liver and beef heart mitochondria. J. Biol. Chem. 267, 26062–26069.

Paulson, R.F., Bernstein, A., 1995. Receptor tyrosine kinases and the regulation of hematopoiesis. Semin. Immunol. 7, 267–277.

Pedemont, C.H., Bertorello, A.M., 2001. Short-term regulation of the proximal tubule Na^+, K^+-ATPase: increased/decreased Na^+, K^+-ATPase activity mediated by protein kinase C isoforms. J. Bioenerg. Biomembr. 33, 439–447.

Pena, C.L., Pendergast, M., Pilar, G., 2002. Selective membrane channels involved in developmental neuronal death. Soc. Neurosci. Abstr. 9, 631.

Penning, L.C., Denecker, G., Vercammen, D., Declercq, W., Schipper, R.G., Vandenabeele, P., 2000. A role for potassium in TNF-induced apoptosis and gene-induction in human and rodent tumour cell lines. Cytokine 12, 747–750.

Pereira, M., Millot, J.M., Sebille, S., Manfait, M., 2002. Inhibitory effects of extracellular Mg^{2+} on intracellular Ca^{2+} dynamic changes and thapsigargin-induced apoptosis in human cancer MCF7 cells. Mol. Cell. Biochem. 229, 163–171.

Perez, G.I., Maravei, D.V., Trbovich, A.M., Cidlowski, J.A., Tilly, J.L., Hughes Jr., F.M., 2000. Identification of potassium-dependent and -independent components of the apoptotic machinery in mouse ovarian germ cells and granulosa cells. Biol. Reprod. 63, 1358–1369.

Perregaux, D., Gabel, C.A., 1994. Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J. Biol. Chem. 269, 15195–15203.

Platia, E.V., Reid, P.R., 1984. Dose-ranging studies of clofilium, an antiarrhythmic quaternary ammonium. Clin. Pharmacol. Ther. 35, 193–202.

Portera-Cailliau, C., Price, D.L., Martin, L.J., 1997. Excitotoxic neuronal death in the immature brain is an apoptosis–necrosis morphological continuum. J. Comp. Neurol. 378, 70–87.

Potter, P.J., Hayes, K.C., Hsieh, J.T., Delaney, G.A., Segal, J.L., 1998. Sustained improvements in neurological function in spinal cord injured patients treated with oral 4-aminopyridine: three cases. Spinal Cord 36, 147–155.

Qin, Z.H., Wang, Y., Chase, T.N., 1996. Stimulation of *N*-methyl-D-aspartate receptors induces apoptosis in rat brain. Brain Res. 725, 166–176.

Quast, U., 1993. Do the K^+ channel openers relax smooth muscle by opening K^+ channels? Trends Pharmacol. Sci. 14, 332–337.

Que, F.G., Gores, G.J., LaRusso, N.F., 1997. Development and initial application of an in vitro model of apoptosis in rodent cholangiocytes. Am. J. Physiol. 272, G106–G115.

Quidt, F.N., 1994. Recording sodium and potassium currents from neuroblastoma cells. In: Ion Channels of Excitable Cells. Academic Press, San Diego, pp. 3–20.

Raff, M.C., Barres, B.A., Burne, J.F., Coles, H.S., Ishizaki, Y., Jacobson, M.D., 1993. Programmed cell death and the control of cell survival: lessons from the nervous system. Science 262, 695–700.

Rajapakse, N., Shimizu, K., Kis, B., Snipes, J., Lacza, Z., Busija, D., 2002. Activation of mitochondrial ATP-sensitive potassium channels prevents neuronal cell death after ischemia in neonatal rats. Neurosci. Lett. 327, 208–212.

Ramsden, M., Plant, L.D., Webster, N.J., Vaughan, P.F., Henderson, Z., Pearson, H.A., 2001. Differential effects of unaggregated and aggregated amyloid β protein (1–40) on K^+ channel currents in primary cultures of rat cerebellar granule and cortical neurones. J. Neurochem. 79, 699–712.

Reeves, W.B., Shah, S.V., 1994. Activation of potassium channels contributes to hypoxic injury in proximal tubules. J. Clin. Invest. 94, 2289–2294.

Repp, H., Nieper, H., Draheim, H.J., Koschinski, A., Muller, H., Dreyer, F., 1998. Infectious bursal disease virus changes the potassium current properties of chicken embryo fibroblasts. Virology 246, 362–369.

Ribera, A.B., Spitzer, N.C., 1992. Developmental regulation of potassium channels and the impact on neuronal differentiation. Ion Channels 3, 1–38.

Robinson, J.D., Flashner, M.S., 1979. The ($Na^+ + K^+$)-activated ATPase. Enzymatic and transport properties. Biochim. Biophys. Acta 549, 145–176.

Rose, C.R., Ransom, B.R., 1996. Intracellular sodium homeostasis in rat hippocampal astrocytes. J. Physiol. (Lond.) 491, 291–305.

Rothman, S.M., Olney, J.W., 1986. Glutamate and the pathophysiology of hypoxic–ischemic brain damage. Ann. Neurol. 19, 105–111.

Rouzaire-Dubois, B., Dubois, J.M., 1998. K^+ channel block-induced mammalian neuroblastoma cell swelling: a possible mechanism to influence proliferation. J. Physiol. (Lond.) 510, 93–102.

Sattler, R., Tymianski, M., 2001. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. Mol. Neurobiol. 24, 107–129.

Schell, S.R., Nelson, D.J., Fozzard, H.A., Fitch, F.W., 1987. The inhibitory effects of K^+ channel-blocking agents on T lymphocyte proliferation and lymphokine production are “nonspecific”. J. Immunol. 139, 3224–3230.

Schlesinger, P.H., Gross, A., Yin, X.M., Yamamoto, K., Saito, M., Waksman, G., Korsmeyer, S.J., 1997. Comparison of the ion channel characteristics of proapoptotic BAX and antiapoptotic BCL-2. Proc. Natl. Acad. Sci. U.S.A. 94, 11357–11362.

Schmidt, T.A., Kjeldsen, K., 1997. Myocardial K homeostasis in ischemia—importance of Na, K-ATPase. Basic Res. Cardiol. 92 (Suppl. 2), 57–59.

Schneggenburger, R., 1998. Altered voltage dependence of fractional Ca^{2+} current in *N*-methyl-D-aspartate channel pore mutants with a decreased Ca^{2+} permeability. Biophys. J. 74, 1790–1974.

Schwarcz, R., Meldrum, B., 1985. Excitatory aminoacid antagonists provide a therapeutic approach to neurological disorders. Lancet 2, 140–143.

Scorrano, L., Petronilli, V., Bernardi, P., 1997. On the voltage dependence of the mitochondrial permeability transition pore. A critical appraisal. J. Biol. Chem. 272, 12295–12299.

Scott, B.S., Fisher, K.C., 1970. Potassium concentration and number of neurons in cultures of dissociated ganglia. Exp. Neurol. 27, 16–22.

Segal, J.L., Brunnemann, S.R., 1997. 4-Aminopyridine improves pulmonary function in quadriplegic humans with longstanding spinal cord injury. Pharmacotherapy 17, 415–423.

Smith-Maxwell, C.J., Eatock, R.A., Begenisich, T., 1991. Induction of K-channel expression in a neuroblastoma cell line. J. Neurobiol. 22, 327–341.

Sonnhof, U., Grafe, P., Krumnikl, J., Linder, M., Schindler, L., 1975. Inhibitory postsynaptic actions of taurine, GABA and other amino acids on motoneurons of the isolated frog spinal cord. Brain Res. 100, 327–341.

Strange, K., 1990. Volume regulation following Na^+ pump inhibition in CCT principal cells: apical K^+ loss. Am. J. Physiol. 258, F732–F740.

Sunderman Jr., F.W., 1995. The influence of zinc on apoptosis. Ann. Clin. Lab Sci. 25, 134–142.

Szucs, A., Stefano, G.B., Hughes, T.K., Rozsa, K.S., 1992. Modulation of voltage-activated ion currents on identified neurons of *Helix pomatia* L. by interleukin-1. Cell. Mol. Neurobiol. 12, 429–438.

Takaba, H., Nagao, T., Yao, H., Kitazono, T., Ibayashi, S., Fujishima, M., 1997. An ATP-sensitive potassium channel activator reduces infarct volume in focal cerebral ischemia in rats. Am. J. Physiol. 273, R583–R586.

Takashi, E., Ashraf, M., 2000. Pathologic assessment of myocardial cell necrosis and apoptosis after ischemia and reperfusion with molecular and morphological markers. J. Mol. Cell. Cardiol. 32, 209–224.

Targ, E.F., Kocsis, J.D., 1985. 4-Aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. Brain Res. 328, 358–361.

Teichberg, V.I., Goldberg, O., Luini, A., 1981. The stimulation of ion fluxes in brain slices by glutamate and other excitatory amino acids. Mol. Cell. Biochem. 39, 281–295.

Tenneti, L., D’Emilia, D.M., Troy, C.M., Lipton, S.A., 1998. Role of caspases in *N*-methyl-D-aspartate-induced apoptosis in cerebrocortical neurons. J. Neurochem. 71, 946–959.

Thompson, C.B., 1995. Apoptosis in the pathogenesis and treatment of disease. Science 267, 1456–1462.

Thompson, G.J., Langlais, C., Cain, K., Conley, E.C., Cohen, G.M., 2001. Elevated extracellular K^+ inhibits death-receptor- and chemical-mediated apoptosis prior to caspase activation and cytochrome *c* release. Biochem. J. 357, 137–145.

Tikhonov, D.B., Zhorov, B.S., Magazanik, L.G., 1999. Intersegment hydrogen bonds as possible structural determinants of the N/Q/R site in glutamate receptors. Biophys. J. 77, 1914–1926.

Trimarchi, J.R., Liu, L., Smith, P.J., Keefe, D.L., 2002. Apoptosis recruits two-pore domain potassium channels used for homeostatic volume regulation. Am. J. Physiol. Cell Physiol. 282, C588–C594.

Tsuzuki, K., Mochizuki, S., Iino, M., Mori, H., Mishina, M., Ozawa, S., 1994. Ion permeation properties of the cloned mouse epsilon 2/zeta 1 NMDA receptor channel. Brain Res. Mol. Brain Res. 26, 37–46.

Ucker, D.S., 1991. Death by suicide: one way to go in mammalian cellular development? New Biol. 3, 103–109.

van Diemen, H.A., Polman, C.H., van Dongen, T.M., van Loenen, A.C., Nauta, J.J., Taphoorn, M.J., van Walbeek, H.K., Koetsier, J.C., 1992. The effect of 4-aminopyridine on clinical signs in multiple sclerosis: a randomized, placebo-controlled, double-blind, cross-over study. Ann. Neurol. 32, 123–130.

van Lookeren Campagne, M., Lucassen, P.J., Vermeulen, J.P., Balazs, R., 1995. NMDA and kainate induce internucleosomal DNA cleavage

associated with both apoptotic and necrotic cell death in the neonatal rat brain. Eur. J. Neurosci. 7, 1627–1640.

Verheye-Dua, F.A., Bohm, L., 2000. Influence of apoptosis on the enhancement of radiotoxicity by ouabain. Strahlenther. Onkol. 176, 186–191.

Walev, I., Reske, K., Palmer, M., Valeva, A., Bhakdi, S., 1995. Potassium-inhibited processing of IL-1 beta in human monocytes. EMBO J. 14, 1607–1614.

Walev, I., Klein, J., Husmann, M., Valeva, A., Strauch, S., Wirtz, H., Weichel, O., Bhakdi, S., 2000. Potassium regulates IL-1 beta processing via calcium-independent phospholipase A2. J. Immunol. 164, 5120–5124.

Wang, X.Q., Yu, S.P., 2002. Tyrosine phosphorylation regulates activity of Na^+, K^+-ATPase in cortical neurons. Soc. Neurosci. Abstr. 8, 446.

Wang, L., Xu, B., White, R.E., Lu, L., 1997. Growth factor-mediated K^+ channel activity associated with human myeloblastic ML-1 cell proliferation. Am. J. Physiol. 273, C1657–C1665.

Wang, L., Xu, D., Dai, W., Lu, L., 1999. An ultraviolet-activated K^+ channel mediates apoptosis of myeloblastic leukemia cells. J. Biol. Chem. 274, 3678–3685.

Wang, X., Xiao, A.Y., Ichinose, T., Yu, S.P., 2000. Effects of tetraethylammonium analogues on apoptosis and membrane currents in cultured cortical neurons. J. Pharmacol. Exp. Ther. 295, 524–530.

Wang, H., Zhang, Y., Cao, L., Han, H., Wang, J., Yang, B., Nattel, S., Wang, Z., 2002. HERG K^+ channel, a regulator of tumor cell apoptosis and proliferation. Cancer Res. 62, 4843–4848.

Wang, X.Q., Xiao, A.Y., Yang, A., LaRose, L., Wei, L., Yu, S.P., 2003a. Block of Na^+, K^+-ATPase and induction of hybrid death by 4-aminopyridine in cultured cortical neurons. J. Pharmacol. Exp. Ther. 305, 502–506.

Wang, X.Q., Xiao, A.Y., Sheline, C., Hyrc, K., Yang, A.Z., Goldberg, M.P., Choi, D.W., Yu, S.P., 2003b. Apoptotic insults impair Na^+, K^+-ATPase activity as a mechanism of neuronal death mediated by concurrent ATP deficiency and oxidant stress. J. Cell Sci. 116, 2099–2110.

Warny, M., Kelly, C.P., 1999. Monocytic cell necrosis is mediated by potassium depletion and caspase-like proteases. Am. J. Physiol. 276, C717–C724.

Watkins, J.C., Evans, R.H., 1981. Excitatory amino acid transmitters. Annu. Rev. Pharmacol. Toxicol. 21, 165–204.

Waxman, S.G., 1993. Aminopyridines and the treatment of spinal cord injury. J. Neurotrauma. 10, 19–24.

Wei, L., Yu, S.P., Gottron, F., Snider, J., Zipfel, G.J., Choi, D.W., 2003. Potassium channel blockers attenuate hypoxia- and ischemia-induced neuronal death in vitro and in vivo. Stroke 34, 1281–1286.

Wesseling, H., Agoston, S., Van Dam, G.B., Pasma, J., DeWit, D.J., Havinga, H., 1984. Effects of 4-aminopyridine in elderly patients with Alzheimer's disease. N. Engl. J. Med. 310, 988–989.

Wible, B.A., Wang, L., Kuryshev, Y.A., Basu, A., Haldar, S., Brown, A.M., 2002. Increased K^+ efflux and apoptosis induced by the potassium channel modulatory protein KChAP/PIAS3beta in prostate cancer cells. J. Biol. Chem. 277, 17852–17862.

Wo, Z.G., Oswald, R.E., 1995. Unraveling the modular design of glutamate-gated ion channels. Trends Neurosci. 18, 161–168.

Wood, M.W., VanDongen, H.M., VanDongen, A.M., 1995. Structural conservation of ion conduction pathways in K channels and glutamate receptors. Proc. Natl. Acad. Sci. U.S.A. 92, 4882–4886.

Xia, S., Lampe, P.A., Deshmukh, M., Yang, A., Brown, B.S., Rothman, S.M., Johnson Jr., E.M., Yu, S.P., 2002. Multiple channel interactions explain the protection of sympathetic neurons from apoptosis induced by nerve growth factor deprivation. J. Neurosci. 22, 114–122.

Xiao, A.Y., Homma, M., Wang, X.Q., Wang, X., Yu, S.P., 2001. Role of K^+ efflux in apoptosis induced by AMPA and kainate in mouse cortical neurons. Neuroscience 108, 61–67.

Xiao, A.Y., Wang, X.Q., Yu, S.P., 2002a. Inhibitory effects of 4-aminopyridine on Na^+, K^+-ATPase and induction of hybrid death in cultured cortical neurons. Soc. Neurosci. Abstr. 10, 701.

Xiao, A.Y., Wang, X.Q., Yang, A., Yu, S.P., 2002b. Slight impairment of Na^+, K^+-ATPase synergistically aggravates ceramide- and β-amyloid-induced apoptosis in cortical neurons. Brain Res. 955, 253–259.

Xiao, A.Y., Wei, L., Xia, S., Rothman, S., Yu, S.P., 2002c. Ionic mechanism of ouabain-induced concurrent apoptosis and necrosis in individual cultured cortical neurons. J. Neurosci. 22, 1350–1362.

Xuan, C.X., Xu, Z.C., 2000. Potassium currents in CA1 neurons of rat hippocampus increase shortly after transient cerebral ischemia. Neurosci. Lett. 281, 5–8.

Yang, A.Z., Wang, X.Q., Yu, S.P., 2002a. Inhibitory effects of clofilium on membrane currents of Ca^{2+} channels, NMDA receptor channels and Na^+, K^+-ATPase in cultured cortical neurons. Soc. Neurosci. Abstr. 11, 701.

Yang, N.C., Jeng, K.C., Ho, W.M., Hu, M.L., 2002b. ATP depletion is an important factor in DHEA-induced growth inhibition and apoptosis in BV-2 cells. Life Sci. 70, 1979–1988.

Yonish-Rouach, E., Grunwald, D., Wilder, S., Kimchi, A., May, E., Lawrence, J.J., May, P., Oren, M., 1993. p53-mediated cell death: relationship to cell cycle control. Mol. Cell. Biol. 13, 1415–1423.

Yoshida, A., Pourquier, P., Pommier, Y., 1998. Purification and characterization of a Mg^{2+}-dependent endonuclease (AN34) from etoposide-treated human leukemia HL-60 cells undergoing apoptosis. Cancer Res. 58, 2576–2582.

Yu, S.P., Choi, D.W., 1999. K^+ efflux mediated by delayed rectifier K^+ channels contributes of neuronal death. In: Alzheimer's Disease and Related Disorders. Wiley, New York, pp. 371–382.

Yu, S.P., Choi, D.W., 2000. Ions, cell volume, and apoptosis. Proc. Natl. Acad. Sci. U.S.A. 97, 9360–9362.

Yu, S.P., Ichinose, T., 1999. The potassium current mediated by NMDA receptor channels in cortical neurons. Soc. Neurosci. Abstr. 25, 1485.

Yu, S.P., Yeh, C.H., Sensi, S.L., Gwag, B.J., Canzoniero, L.M., Farhangrazi, Z.S., Ying, H.S., Tian, M., Dugan, L.L., Choi, D.W., 1997. Mediation of neuronal apoptosis by enhancement of outward potassium current. Science 278, 114–117.

Yu, S.P., Farhangrazi, Z.S., Ying, H.S., Yeh, C.H., Choi, D.W., 1998. Enhancement of outward potassium current may participate in β-amyloid peptide-induced cortical neuronal death. Neurobiol. Dis. 5, 81–88.

Yu, S.P., Yeh, C., Strasser, U., Tian, M., Choi, D.W., 1999a. NMDA receptor-mediated K^+ efflux and neuronal apoptosis. Science 284, 336–339.

Yu, S.P., Yeh, C.H., Gottron, F., Wang, X., Grabb, M.C., Choi, D.W., 1999b. Role of the outward delayed rectifier K^+ current in ceramide-induced caspase activation and apoptosis in cultured cortical neurons. J. Neurochem. 73, 933–941.

Yu, S.P., Canzoniero, L.M., Choi, D.W., 2001. Ion homeostasis and apoptosis. Curr. Opin. Cell Biol. 13, 405–411.