

Review

Potassium Efflux During Apoptosis

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Introduction

Apoptosis is responsible for normal tissue homeostasis and is known to mediate pathological cell loss (Fulton, 1996; Nagata, 1997; Salvesen and Dixit, 1997; Wallach, 1997; Wyllie, 1998). Apoptosis is characterized by morphological changes. These include cell body and nuclear breakdown, chromatin condensation and fragmentation, and formation of apoptotic bodies (Erhardt and Cooper, 1996; Kothakota *et al.*, 1997; Liu *et al.*, 1997; Enari *et al.*, 1998; Janicke *et al.*, 1998a, b; Zheng *et al.*, 1998; McLroy *et al.*, 1999; Sakahira *et al.*, 1999; Zhang *et al.*, 1999; D'Mello *et al.*, 2000). Recently, molecular details of apoptosis was elucidated, and apoptotic genes and proteins were increasingly characterized (Du *et al.*, 2000; Srinivasula *et al.*, 2000; Wu *et al.*, 2000). Several apoptotic-signaling pathways have been suggested. Receptor-mediated apoptosis is characterized by the formation of the death-inducing signaling complex (DISC), comprising the Fas receptor, FADD, FLASH, procaspase-8 and other proteins (Minn *et al.*, 1996; Muzio *et al.*, 1996; Stennicke and Salvesen, 1997; Chen *et al.*, 1998; Widmann *et al.*, 1998; Yeh *et al.*, 1998). Procaspase-8 is processed to active caspase-8, which in turn cleaves downstream signaling proteins like procaspase-3. On the other hand, chemical-induced apoptosis generally leads to mitochondrial damage and the subsequent release of cytochrome c (Krippner *et al.*, 1996; Chauhan *et al.*, 1997; Kluck *et al.*, 1997; Yang *et al.*, 1997; Eskes *et al.*, 1998; Bossy-Wetzels and Green, 1999; Fiers *et al.*, 1999; Granville *et al.*, 1998; Gross *et al.*, 1999; Kluck *et al.*, 1999; Martinou, 1999; Schapira, 1999). This protein associates with Apaf-1, caspase-9 and dATP to form a multiprotein complex called the apoptosome (Li *et al.*, 1997; Yang *et al.*, 1997; Zou *et al.*, 1997; Srinivasula *et al.*, 1998; Chinnaiyan, 1999; Fujita *et al.*, 1999; Hu *et al.*, 1999; Krajewski *et al.*, 1999; Stennicke *et al.*,

1999; Zou *et al.*, 1999; Cain *et al.*, 2000; Purring-Koch and McLendon, 2000). This complex acts as a kind of holoenzyme that activates procaspase-3 and -7. DFF45/ICAD is cleaved by active caspase-3 to release its complexed DNase, DFF40/CAD, which in turn degrades nuclear DNA (Liu *et al.*, 1998; Zheng *et al.*, 1998; Liu *et al.*, 1999; McCarty *et al.*, 1999; Wolf *et al.*, 1999). Caspase-3 is also responsible for the processing of Acinus, a chromosomal condensation factor (Sahara *et al.*, 1999). A cross-talk exists between the two apoptotic pathways, which are not exclusive of each other. Caspase-8 cleaves Bid, which in turn acts on mitochondria to release cytochrome c (Scaffidi *et al.*, 1997; Juo *et al.*, 1998; Luo *et al.*, 1998; Srinivasan *et al.*, 1998; Gross *et al.*, 1999; Kluck *et al.*, 1999; Lin *et al.*, 1999; Sun *et al.*, 1999; Tan *et al.*, 1999; Zhuang *et al.*, 1999; Liu *et al.*, 2000). Conversely, caspase-3 and other downstream caspases activate procaspase-8 (Stennicke and Salvesen, 1997; Stennicke *et al.*, 1998). The requirement for this activation is unclear, but possibly occurs to induce amplification of the apoptotic signal. Recently, caspase-12 was identified on endoplasmic reticulum (ER) (Nakagawa *et al.*, 2000). The activation pathway of the caspase was mediated by m-calpain, which suggests that the change of the intracellular calcium concentration may be the triggering signal. The caspase was also cleaved and activated by caspase-7. This observation provides the link between chemical or receptor-mediated and ER-mediated apoptosis. However, until recently none of the caspase-12 substrates were identified, except the caspase itself. How the caspase induces apoptosis remains to be elucidated.

During apoptosis, several physiological changes are observed. It is known that the pH in cells that undergo apoptosis drops. It is also known that calcium influx occurs and potassium and chloride ions efflux in cells that undergo apoptosis (Bortner *et al.*, 1997; Yu *et al.*, 1997; Bortner and Cidlowski, 1999). However, the molecular details of the physiological changes are largely unknown. Recently several reports have indicated the importance of the changes for ensuring cell death. Here, the relationship between potassium efflux and apoptosis is reviewed.

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Potassium efflux during apoptosis

Potassium is a major intracellular ion. Several lines of studies indicate that the enhancement of potassium current is directly involved in apoptosis (Bortner *et al.*, 1997; Yu *et al.*, 1997; Dallaporta *et al.*, 1998; Hughes *et al.*, 1997; Orlov *et al.*, 2000; Shieh *et al.*, 2000). In the neuronal cell, the tetraethylammonium (TEA)-sensitive potassium current was enhanced by serum deprivation or staurosporine. Inhibition of outward potassium currents with TEA or elevated extracellular potassium, but not with blockers of chloride or other potassium channel blockers, did reduce apoptosis. It is also shown that exposure to the potassium ionophore valinomycin induced apoptosis. In thymocyte, apoptosis that was induced by dexamethason, etoposide, or ceramide was also repressed by the potassium channel blocker TEA (Franklin *et al.*, 1995; Hughes *et al.*, 1997; Hughes and Cidlowski, 1999; Yu *et al.*, 1999). The tumor necrosis factor (TNF) also had an effect on the ion channel opening and death in the rat liver cell line. TNF elicited 2- and 5- fold increases in potassium and chloride current. The activation of the potassium channel may be an early response to TNF signaling (Kampf *et al.*, 1999; Nietsch *et al.*, 2000; Penning *et al.*, 2000). As a result of the potassium efflux, the concentration decreases from ~140 mM, the normal physiological concentration to 30~50 mM.

Signal transduction related with potassium efflux

The loss of the intracellular potassium during apoptosis is an early requisite feature, although how it is regulated on the apoptotic signal is unknown. One report provided evidence that the protein kinase C (PKC) may be involved in the process (Gomez-Angelats *et al.*, 2000; Nietsch *et al.*, 2000). Anti-Fas induced cell shrinkage is thought to be a result of the loss of the intracellular potassium that was blocked by PKC stimulation. Conversely, the inhibition of PKC enhanced the anti-Fas-mediated loss of cell volume. From this result, it was proposed that PKC could regulate the ion channel and block the initial loss of intracellular potassium and cell shrinkage. PKC is a well-known substrate of caspase. Also, the stimulation of PKC prevented the cleavage of PKC during apoptosis. Although there is no evidence yet, observations suggest that the initial apoptotic pathway propagates and results in the activation of caspase, which in turn inactivates PKC by cleavage to unlock the inhibitory role of the kinase on the potassium channel.

Inhibition of apoptosome formation by intracellular potassium

Stress-induced apoptotic cell death is triggered by the release of the mitochondrial cytochrome c (Kluck *et al.*, 1997; Vier *et*

al., 1999). The mitochondrial protein (together with Apaf-1 and dATP) induces the processing of caspase-9 and initiates the caspase cascade (Li *et al.*, 1997; Yang *et al.*, 1997; Zou *et al.*, 1997; Srinivasula *et al.*, 1998; Chinnaiyan, 1999; Fujita *et al.*, 1999; Hu *et al.*, 1999; Krajewski *et al.*, 1999; Stennicke *et al.*, 1999; Zou *et al.*, 1999; Cain *et al.*, 2000; Purring-Koch and McLendon, 2000). Apaf-1, the first identified mammalian homolog of CED-4, is an ~130-kDa that contains an N-terminal caspase recruitment domain, a CED-4 homology region, and WD-40 repeats. Recent studies indicate that in the presence of cytochrome c and dATP/ATP, Apaf-1 undergoes oligomerization to form large apoptosome complexes with molecular masses of 700~1400 kDa (Cain *et al.*, 2000; Hausmann *et al.*, 2000; Purring-Koch and McLendon, 2000). The apoptosome complex recruits and activates procaspase-9, which forms a holoenzyme complex. As a result, caspase-9 is activated and processes the downstream caspase.

Initial studies indicate that the interaction of purified cytochrome c with Apaf-1 strongly depends on the ionic strength. Two cytochromes c bind to one Apaf-1 with K_a at zero NaCl of approximately 10^{10} M^{-1} that decreased to K_a at 200 mM NaCl of approximately 10^7 M^{-1} . Recently, another line of study demonstrates that the assembly of the active complex is suppressed by normal intracellular concentrations of potassium (~150 mM). Using a defined apoptosome reconstitution system with recombinant Apaf-1 and cytochrome c, potassium also inhibited caspase activation by abrogating the apoptosome formation. This study suggests that the normal intracellular concentration of potassium suppresses the cell death by inhibiting the formation of the Apaf-1 apoptosome complex.

One question regarding this is whether the release of cytochrome c from the mitochondria during stress-induced apoptosis occurs in the presence of the intracellular potassium. One clue from our studies is that the apoptosis, which is induced by staurosporine in the neuroblastoma cell line and HeLa cell, was significantly inhibited by ~150 mM potassium in the media, while the release of cytochrome c still occurs (unpublished result). Consistent with the inhibition of the apoptosome formation by 150 mM potassium in the reconstituted experiment, the protein complex was not formed. This indicates that the inhibitory effect of the intracellular potassium on the apoptosis may have an effect that is mainly on the formation of the protein complex. This result is compatible with the suggestion that for the apoptosis to occur through the mitochondrial pathway, the intracellular potassium should be removed by a mechanism that has not yet been identified. Other groups, however, reported that the cytochrome c release from mitochondria during apoptosis is greatly reduced in the presence of a high concentration of potassium (Maeno *et al.*, 2000). These controversial observations may reflect the complexity and diversity of the inhibitory mechanism of potassium against apoptosis.

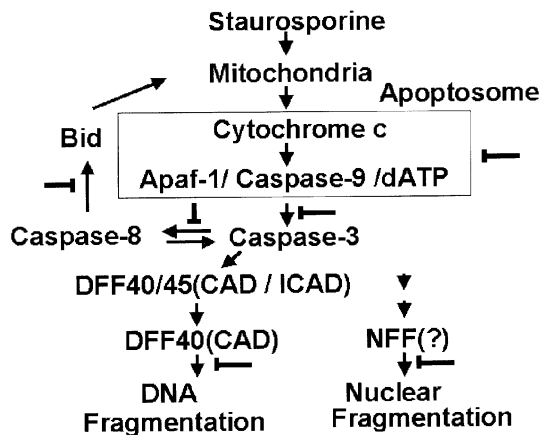


Fig. 1. Proposed model for the negative regulation of apoptosis by intracellular high potassium. NFF, Nuclear fragmentation factor.

The effect of potassium on the downstream of the Apaf-1 apoptosome formation

Potassium also inhibits apoptotic DNA fragmentation, one of the hallmarks of apoptosis (Hughes *et al.*, 1997; Bortner and Cidlowski, 1999; D'Mello *et al.*, 2000). In the cell-free system experiment with the apoptotic cell extract and isolated nuclei, DNA fragmentation was significantly inhibited by potassium at a high concentration. DFF40/CAD is the main DNase that is responsible for the DNA fragmentation, however, which DNase is directly influenced by the ion is unknown. In our experiment, purified CAD showed decreased activity under a high concentration (>100 mM) of potassium. This is consistent with the inhibitory effect of potassium on the DNA fragmentation (unpublished results). Potassium, however, did not inhibit the activity of caspase-3 once activated. Nuclear fragmentation and chromatin condensation are also important hallmarks of apoptosis. In many cases, DNA fragmentation is accompanied by nuclear fragmentation and chromatin condensation; however, it is reported that nuclear fragmentation and chromatin condensation could occur without DNA fragmentation (Sahara *et al.*, 1999). One candidate that is responsible for the apoptotic chromatin condensation is Acinus. When it is used in a cell free system, the chromatin condensation and nuclear fragmentation that are independent of DNA fragmentation are shown to be also significantly suppressed by potassium at more than 100 mM (unpublished result).

Conclusion

The blockage of potassium loss by a high concentration of extracellular potassium repressed apoptosis. When sympathetic neurons were maintained in a suboptimal concentration of neurotrophin, the coincident depolarization with concentrations of KCl, which on their own had no

survival effect, synergistically enhanced survival (Vaillant *et al.*, 1999). This may be attributed to the activation of the Ras-phosphatidylinositol 3-kinase-Akt pathway. This illustration may be helpful in the prevention or cure of some degenerative diseases, such as Alzheimers disease.

As potassium efflux is an essential part of apoptosis, the existence of the signal to the potassium channel is evident, although it remains to be elucidated. Uncovering the pathway will greatly increase our understanding of the important physiological process. In summary, apoptotic pathways that are sensitive to intracellular potassium or blocked by inhibition potassium efflux are indicated (observations in Figure 1).

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