Attenuation of apoptosis in enterocytes by blockade of potassium channels

Anatoly Grishin,1 Henri Ford,1 Jin Wang,1 Hui Li,2 Vicenta Salvador-Recatala,2 Edwin S. Levitan,2 and Elena Zaks-Makhina2

1Division of Pediatric Surgery, Children’s Hospital of Los Angeles, Los Angeles, California; and 2Department of Pharmacology, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania

Submitted 4 January 2005; accepted in final form 29 June 2005

Grishin, Anatoly, Henri Ford, Jin Wang, Hui Li, Vicenta Salvador-Recatala, Edwin S. Levitan, and Elena Zaks-Makhina. Attenuation of apoptosis in enterocytes by blockade of potassium channels. Am J Physiol Gastrointest Liver Physiol 289: G815–G821, 2005. First published July 28, 2005; doi:10.1152/ajpgi.00001.2005.—Apoptosis plays an important role in maintaining the balance between enterocyte proliferation and cell loss in the intestinal epithelium. Apoptosis rates may increase in intestinal pathologies such as inflammatory bowel disease and necrotizing enterocolitis, suggesting pharmacological prevention of apoptosis as a therapy for these conditions. Here, we explore the feasibility of this approach using the rat epithelial cell line IEC-6 as a model. On the basis of the known role of K+ efflux in apoptosis in various cell types, we hypothesized that K+ efflux is essential for apoptosis in enterocytes and that pharmacological blockade of this efflux would inhibit apoptosis. By probing intracellular [K+] with the K+-sensitive fluorescent dye and measuring the efflux of 86Rb+, we found that apoptosis-inducing treatment with the proteasome inhibitor MG-132 leads to a twofold increase in K+ efflux from IEC-6 cells. Blockade of K+ efflux with tetraethylammonium, 4-aminopyridine, stromatoxin, chromanol 293B, and the recently described K+ channel inhibitor 48F10 prevents DNA fragmentation, caspase activation, release of cytochrome c from mitochondria, and loss of mitochondrial membrane potential. Thus K+ efflux occurs early in the apoptotic program and is required for the execution of later events. Apoptotic K+ efflux critically depends on activation of p38 MAPK. These results demonstrate for the first time the requirement of K+ channel-mediated K+ efflux for progression of apoptosis in enterocytes and suggest the use of K+ channel blockers to prevent apoptotic cell loss occurring in intestinal pathologies.

IEC-6 cells; apoptotic K+ efflux; p38 mitogen-activated protein kinase; intestinal pathologies

In the intestinal epithelium, constant renewal of enterocytes is essential for maintaining tissue homeostasis. Epithelial stem cells in intestinal crypts divide and differentiate into enterocytes that migrate to villus tips, where they ultimately undergo apoptosis (29). The balance between cell proliferation and cell loss in crypts (11, 23, 39) and villi (19, 39) is controlled by apoptosis. Under pathological conditions, the equilibrium between enterocyte proliferation and apoptosis may shift toward the latter process. Dysregulated or accelerated apoptosis as seen in several forms of inflammatory bowel disease (21), bowel injury (14), and infection (17, 35) may result in gut barrier failure. Thus pharmacological manipulation or control of epithelial apoptosis may be a useful therapeutic modality for intestinal pathologies associated with excessive apoptosis.

Apoptosis is programmed cell death in response to extrinsic and intrinsic cues such as death receptor ligands or stresses. Although apoptosis is irreversible, its later steps such as caspase substrate cleavage, DNA fragmentation, and dismantling of cellular structures can be prevented by blocking the earlier steps such as mitochondrial depolarization, cytochrome c release, and activation of caspases. Recently, perturbations in ion homeostasis have been discovered as important steps in the apoptotic program (41). Dramatic efflux of K+ occurs shortly after apoptotic stimulation and results in a significant decrease in intracellular [K+] (42). Importantly, blockade of K+ efflux by elevated external [K+] or K+ channel blockers prevents later stages of apoptosis (27, 37, 43). These findings suggest that K+ efflux via K+ channels is an essential step of the apoptotic program that may present a valid target for pharmacological modulation of apoptosis. Involvement of K+ currents in apoptosis has been demonstrated in neurons (26, 27, 42), lymphocytes (7, 13, 16), smooth muscle cells (5, 20), cardiac cells (6), and various cell lines (40) but not in enterocytes.

If K+ currents are important for apoptotic processes in enterocytes, attenuation of apoptosis by K+ channel block may be beneficial for treatment of intestinal disorders associated with increased enterocyte apoptosis. As a first step toward developing this therapeutic approach, we examined whether apoptotic insults evoke K+ efflux and whether blockade of K+ efflux attenuates apoptosis in IEC-6 enterocytes. As a model cytoprotective drug, we used 3-bicyclo(2.2.1)hept-2-yl-benzene-1,2-diol (48F10), a compound that we have previously identified as a K+ channel blocker with high antiapoptotic efficacy in neurons (45). In this study, we demonstrated the importance of K+ efflux for the progression of apoptosis in IEC-6 enterocytes and the prevention of apoptotic cell loss by pharmacological blockade of K+ channels.

MATERIALS AND METHODS

Cell culture. IEC-6 cells were purchased from American Type Culture Collection (ATCC; Rockville, MD). Cells were grown in DMEMMM (Bio-Whittaker; Walkersville, MD) with 4.5 g/l glucose and 0.02 mM glutamine, supplemented with 5% fetal bovine serum (Bio-Whittaker), 0.1 U/ml insulin, and 100 μg/ml penicillin-100 μg/ml streptomycin at 37°C and 10% CO2. Passages 17–27 were used. CHO cells obtained from ATCC were grown in media of the same composition as above but without insulin. CHO cells were incubated at 37°C and 5% CO2.

Induction of apoptosis. Subconfluent cultures were treated with 5 μM MG-132 (BioMol; Plymouth Meeting, PA) in growth medium. For peroxynitrite treatment, cells were washed with PBS, incubated for 15 min in 50 μM peroxynitrite (Alexis Biochemical; San Diego, CA) in PBS (155.17 mM NaCl, 2.97 mM Na2HPO4, and 1.06 mM KH2PO4; pH 7.2), and then transferred to growth medium. Control cells were treated with equivalent amounts of DMSO or decomposed peroxynitrite. Cells were harvested 4–20 h posttreatment.

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Measurement of intracellular \(K^+\). Intracellular \(K^+\) content was assessed using the cell-permeant AM derivative of a \(K^+\) binding fluorescent indicator (PBFI-AM; Molecular Probes; Eugene, OR) as recommended by the manufacturer. Cells were grown in 10-cm petri dishes with 5 \(\mu\)M MG-132 for 7 h, trypsinized, washed with PBS, and resuspended in 1 ml of media containing 10 \(\mu\)M PBFI-AM for dye loading. PBFI-AM was prepared by mixing 10 mM PBFI-AM in DMSO with 25% Pluronic-127 (Molecular Probes) in DMSO. Before cells were loaded, cell density in all samples was measured using a hemocytometer and equalized, if necessary, by dilution with PBS. Cells were incubated in dye-containing media for 1 h at 37°C and 10% \(CO_2\). After being loaded, cells were washed twice with PBS, pelleted by centrifugation at 400 \(g\) for 10 min at 4°C. The supernatant was adjusted with 1% SDS and 20 mM Tris (pH 7.5). The cell suspension was incubated with 50 mM Triton X-100 for 4 h during incubation with 1 \(\mu\)Ci/ml \(^{86}\text{Rb}^+\) (Amersham Biosciences; Piscataway, NJ). During the \(^{86}\text{Rb}^+\) loading, cells were induced to undergo apoptosis and analyzed for \(^{86}\text{Rb}^+\) efflux 2 and 5 h after induction. Before \(^{86}\text{Rb}^+\) measurements, cells were washed three times with \(^{86}\text{Rb}^+\)-free media. Media were collected at 0- to 2-h time points, and the amount of released \(^{86}\text{Rb}^+\) was measured by scintillation counting. At 2 h, cells were lysed with 1% SDS, and the residual radioactivity in cells was determined.

Cell toxicity assay. Cell death was assessed visually at \(\times40\) magnification using the lactate dehydrogenase (LDH)-based toxicity assay kit (Sigma-Aldrich; St. Louis, MO) according to the manufacturer’s instructions.

DNA fragmentation assay. Cells were grown in 10-cm petri dishes. Ten hours after the induction of apoptosis, cells were lysed for 10 min on ice with the DNA ladder buffer [20 mM Tris (pH 7.5), 10 mM EDTA, 100 mM NaCl, and 0.5% Triton X-100]. The lysate was cleared by centrifugation at 14,000 \(g\) for 10 min at 4°C. The supernatant was adjusted with 1% SDS and 20 \(\mu\)g/ml proteinase K (Pierce Biotechnology; Rockford, IL) and incubated at 45°C for 4 h. The sample was extracted with phenol-chloroform, treated with RNase A (50 \(\mu\)g/ml), and precipitated with 2 volumes of ethyl alcohol. DNA was analyzed on 2% agarose gel stained with ethidium bromide.

Caspase activity assay. Cells with active caspases were detected 10 h after the induction of apoptosis by staining with FAM-VAD-FMK (Pharmingen; San Diego, CA), caspase 3 (Stressgen; San Diego, CA), and p38 MAPK and phosphorylated p38 (Cell Signaling Technology; Beverly, MA) were used for Western blot analysis as directed by their manufacturers. Secondary horseradish peroxidase-conjugated antibodies were purchased from Bio-Rad Laboratories (Hercules, CA).

Chemicals. The \(K^+\) channel blocker 3-bicyclo(2.2.1)hept-2-yl-benzene-1,2-dioli (48F10; identification no. 5115671) was purchased from ChemBridge (San Diego, CA). Chromanol 293B was purchased from Sigma-Aldrich, and the efflux of radioactivity was measured 6 h after treatment using the \(K^+\) channel inhibitor 48F10.

RESULTS

Inducers of apoptosis cause \(K^+\) efflux in IEC-6 cells. An increase in \(K^+\) efflux has been identified as an essential early step of apoptosis in several tissues and cell lines. To examine whether \(K^+\) efflux occurs during apoptosis in enterocytes, we measured \(K^+\) efflux in the IEC-6 rat intestinal epithelial cell line after apoptosis-inducing treatment. The IEC-6 cell line was chosen because it is a nontransformed primary enterocyte cell line that is likely to possess the wild-type complement of all apoptotic genes. Logarithmically growing IEC-6 cells were treated with the proteasome inhibitor MG-132, which induces apoptosis via protein denaturing stress. Levels of intracellular \(K^+\) were measured 6 h after treatment using the \(K^+\)-sensitive fluorescent dye PBFI. Figure 1, A and B, shows that cells treated with MG-132 retain less \(K^+\) than control cells. The loss of intracellular \(K^+\) was inhibited if the \(K^+\) channel inhibitor 48F10 was present in media during treatment with MG-132 (Fig. 1, A and B). To corroborate the data obtained with the PBFI dye and to determine the time course of \(K^+\) efflux, we used the \(^{86}\text{Rb}^+\) flux assay, which is based on the similar selectivity of \(K^+\) channels toward \(K^+\) and \(Rb^+\). Cells were preloaded with \(^{86}\text{Rb}^+\), and the efflux of radioactivity was measured 2 and 5 h after treatment with MG-132 (Fig. 1, C and D). As shown in Fig. 1D, \(^{86}\text{Rb}^+\) efflux was similar in MG-132-treated and control cells 2 h after treatment. However, at 5 h, \(^{86}\text{Rb}^+\) efflux occurred faster from MG-132-treated cells (Fig. 1, C and D). 48F10 at 10 \(\mu\)M significantly reduced the \(^{86}\text{Rb}^+\) efflux evoked by MG-132 (Fig. 1, C and D). \(^{86}\text{Rb}^+\) efflux in control cells was insensitive to 48F10 at 10 \(\mu\)M (Fig. 1D).
sequence of apoptosis or cell damage. To distinguish between a step in the apoptotic process. Alternatively, it may be a consequence after treatment with an inducer of apoptosis may be an early presence of 48F10, both LDH release and DNA fragmentation treatment with peroxynitrite. Similar results were obtained was assessed visually as cell shrinkage and detachment as well as the percentage of LDH release from untreated cells and treated with apoptosis inducers in the presence and absence of 10 μM 48F10. Data are presented as the percentage of LDH release from untreated cells in the absence of 48F10. Bars represent means ± SE from 3 independent experiments.

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Fig. 1. Apoptotic inducer MG-132 evokes K⁺ efflux via K⁺ channels. A: emission spectra of PBFI-loaded cells treated for 7 h with DMSO solvent, 5 μM MG-132, and 5 μM MG-132 plus 10 μM 48F10. Open symbols, spectra emitted by PBFI in complex with K⁺ after excitation at 340 nm; solid symbols, spectra of total PBFI in the same samples after excitation at 380 nm (control for dye loading); F, fluorescence intensity. B: relative fluorescence of PBFI-loaded cells in the presence and absence of 10 μM 48F10. Bars represent means ± SE of 3 independent experiments. C: time course of ⁸⁶Rb⁺ efflux. Cells were loaded with ⁸⁶Rb⁺ and treated with DMSO [control (Con)], 5 μM MG-132, 5 μM MG-132 plus 10 μM 48F10, or 10 μM 48F10 for 5 h. Cells were washed, and ⁸⁶Rb⁺ release was measured at the indicated time points. D: ⁸⁶Rb⁺ efflux at 2 and 5 h after induction of apoptosis. Cells were loaded with ⁸⁶Rb⁺ and treated with 5 μM MG-132 or 5 μM MG-132 plus 10 μM 48F10 for 2 or 5 h. ⁸⁶Rb⁺ released from cells was measured 25 min after cells were washed. Data shown are means ± SE of 3 independent experiments.

1, C and D) and 50 μM (not shown). These data indicate that treatment of IEC-6 cells with the apoptosis-inducing agent MG-132 leads to K⁺ efflux via 48F10-sensitive K⁺ channels.

Blockade of K⁺ efflux has a cytoprotective effect. K⁺ efflux after treatment with an inducer of apoptosis may be an early step in the apoptotic process. Alternatively, it may be a consequence of apoptosis or cell damage. To distinguish between these two possibilities, we examined the effects of blockade of K⁺ efflux with 48F10 on cell death and apoptosis. Cell death was assessed visually as cell shrinkage and detachment as well as by LDH release, a consequence of plasma membrane rupture. Apoptosis was assessed by internucleosomal DNA fragmentation. Figure 2A shows that 48F10 reduces cell death upon treatment with peroxynitrite. Similar results were obtained when apoptosis was induced by MG-132 (not shown). In the presence of 48F10, both LDH release and DNA fragmentation were significantly attenuated in cells treated to undergo apoptosis (Fig. 2, B and C). These data argue that K⁺ efflux is a prerequisite and not a consequence of cell death after apoptosis-inducing treatments.

Blockade of K⁺ efflux stops the apoptotic program at its early steps. The fact that K⁺ efflux blockade prevents DNA fragmentation, a late step of apoptosis, may indicate that lowering intracellular [K⁺] is required for the progression of apoptosis at this or an earlier stage. To identify the stage of apoptosis requiring K⁺ efflux, we examined whether execution of various events of the apoptotic program, including caspase activation, cytochrome c release from mitochondria, and changes in mitochondrial membrane potential, can be blocked by a K⁺ current inhibitor.

Activation of caspases (apoptotic proteases) was assayed using the fluorescent pan-caspase inhibitor FAM-VAD-FMK. This agent binds to mature active caspases but not to their precursors. Figure 3A shows that MG-132 dramatically increases the fraction of FAM-VAD-FMK-positive cells 12 h posttreatment, indicating caspase activation. 48F10 attenuated this effect in a concentration-dependent manner (Fig. 3B). At 10 μM, 48F10 reduced the frequency of apoptotic cells to near-background levels. 48F10 did not interfere with FAM-VAD-FMK staining because it did not reduce the percentage of positive cells when added simultaneously with FAM-VAD-FMK (data not shown). Similar results were obtained when
MG-132-induced K\(^+\) efflux was prevented by other inhibitors of K\(^+\)/H\(_{11001}\) currents, including tetraethylammonium (TEA), 4-aminopyridine (4-AP), and elevated external [K\(^+\)] (Fig. 3C). The effect of K\(^+\)/H\(_{11001}\) current blockade on caspase activation was also examined by Western blot analysis with the antibody that recognizes both active caspase 3 (15 kDa) and its inactive precursor (25 kDa) (Fig. 4). 48F10 inhibited caspase 3 activation by MG-132 in a concentration-dependent manner (Fig. 4, A and B). 48F10 also inhibited caspase 3 activation by peroxynitrite (Fig. 4, C and D). These results indicate that blockade of K\(^+\)/H\(_{11001}\) efflux inhibits apoptosis at a stage upstream of caspase activation.

We next examined effects of 48F10 on cytochrome c release from mitochondria, an event in the apoptotic cascade that occurs before caspase activation. Treatment with MG-132 caused the release of cytochrome c from mitochondria into the cytosol (Fig. 5A). Twelve hours posttreatment, about 80% of cytochrome c partitioned into the cytosol compared with <5% in control cells. In the presence of 48F10, only about 10% of cytochrome c became cytosolic after MG-132 treatment. These data indicate that K\(^+\) efflux occurs before the release of cytochrome c from mitochondria.

The release of cytochrome c occurs after the loss of mitochondrial membrane potential. To test whether blockade of K\(^+\) efflux prevents deenergization of mitochondria, we assessed changes in mitochondrial membrane potential using the fluorescent Mitosensor dye. This dye accumulates in energized but not deenergized mitochondria. Treatment with MG-132 caused a significant decrease in the fraction of cells displaying fluorescent mitochondria, which is indicative of apoptosis (Fig. 5B). 48F10 reversed this effect (Fig. 5B). This result indicates that MG-132-induced K\(^+\) efflux was prevented by other inhibitors of K\(^+\) currents, including tetraethylammonium (TEA), 4-aminopyridine (4-AP), and elevated external [K\(^+\)] (Fig. 3C). The effect of K\(^+\) current blockade on caspase activation was also examined by Western blot analysis with the antibody that recognizes both active caspase 3 (15 kDa) and its inactive precursor (25 kDa) (Fig. 4). 48F10 inhibited caspase 3 activation by MG-132 in a concentration-dependent manner (Fig. 4, A and B). 48F10 also inhibited caspase 3 activation by peroxynitrite (Fig. 4, C and D). These results indicate that blockade of K\(^+\) efflux inhibits apoptosis at a stage upstream of caspase activation.

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**Apoptotic K⁺ efflux is mediated by voltage-gated K⁺ channels.** The fact that 48F10 and TEA, known blockers of Kv2.1 and KvLQT1 channels (12), prevent MG-132-induced apoptosis may indicate that these channels are involved in apoptotic K⁺ loss in IEC-6 cells. Kv2.1 and KvLQT1 channels were expressed in MG-132-treated IEC-6 cells at the mRNA level, as judged by RT-PCR (Fig. 6A). To further examine the roles of these channels in apoptotic K⁺ efflux, we tested the antiapoptotic effects of stromatoxin and chromanol 293B, which have been reported to specifically inhibit Kv2.1 and KvLQT1, respectively (31, 9). Both inhibitors decreased the percentage of cells with active caspases after treatment with MG-132, supporting the roles of Kv2.1 and KvLQT1 in apoptosis (Fig. 6B). Moreover, stromatoxin at 40 nM and chromanol 293B at 20 μM concentrations decreased the frequency of cells with deenergized mitochondria by half, as judged by MitoSensor staining (not shown). In control experiments, we tested the selectivity of 48F10, chromanol 293B, and stromatoxin toward Kv2.1 and KvLQT1 currents in transfected CHO cells. Figure 6C shows that chromanol 293B inhibits both KvLQT1 and Kv2.1 currents, whereas stromatoxin affects Kv2.1 only. Taken together, these data point to the Kv2.1 channel as a mediator of apoptotic K⁺ efflux in IEC-6 cells but do not exclude involvement of the KvLQT1 channel.

![Image](image1.png)

**Fig. 6. Identification of proapoptotic K⁺ channels.** A: products of RT-PCR of total RNA from IEC-6 cells treated for 6 h with MG-132. The template was amplified by PCR with Kv2.1 and KvLQT1 primers. The identity of PCR products was verified by sequencing. B: concentration-dependent inhibition of apoptosis by chromanol 293B and stromatoxin (ScTx). Cells were treated with 5 μM MG-132 for 10 h with or without the inhibitors at the indicated concentrations (μM for chromanol 293B and nM for ScTx). Apoptotic cells were scored by FAM-VAD-FMK fluorescence. Apoptosis efficiency is expressed relative to MG-132 treatment without inhibitors, which was assumed 100%. Bars are means ± SE; n = 3 experiments. C: representative whole cell K⁺ current recordings from CHO cells transfected with Kv2.1 and KvLQT1 cDNAs in the presence and absence of K⁺ channel blockers (concentrations of 48F10 and chromanol 293B are in μM, stromatoxin is in nM). K⁺ currents were elicited by pulses to +60 mV, delivered every 10 s, from a holding potential of −70 mV.

**p38 MAPK is implicated in the activation of apoptotic K⁺ efflux.** On the basis of the known role of p38 MAPK in the apoptotic response in various cell types (1, 24, 34, 36), we hypothesized that p38 is involved in the regulation of apoptotic

![Image](image2.png)

**Fig. 7. p38 MAPK mediates MG132-induced apoptosis.** A: time-dependent activation of p38 by MG-132. Cells were treated with 5 μM MG-132 for the indicated times (in min). Activating phosphorylation of p38 was detected by Western blot analysis with phospho-specific antibody (top). **Bottom,** reprobe of the same blot with regular p38 antibody. B: the specific inhibitor of p38 blocks MG-132-induced procaspase-3 cleavage. IEC-6 cells were pretreated with or without 20 μM SB-202180 for 20 min and then treated with MG-132 for 6 h. Caspase-3 cleavage was examined by Western blot analysis with anti-caspase-3 antibody. Active caspase appears as a 15-kDa band. C: the specific inhibitor of p38 attenuates MG-132-induced apoptosis. IEC-6 cells were pretreated with or without 20 μM SB-202180 for 20 min and then treated with 5 μM MG-132 for 12 h. Apoptosis was scored as a percentage of FAM-VAD-FMK-positive cells. Spontaneous apoptosis. Bars are means ± SE; n = 3 experiments. *P < 0.05.

**Fig. 8. p38 MAPK mediates apoptotic K⁺ efflux.** A: time course of ⁸⁶Rb⁺ efflux from MG-132-treated cells in the presence and absence of the p38 inhibitor SB-202180. IEC-6 cells were loaded with ⁸⁶Rb⁺ for 4 h. Cells were then pretreated with or without 20 μM SB-202180 for 20 min, and apoptosis was induced by treatment with 5 μM MG-132 for 5 h. Cells were washed, and ⁸⁶Rb⁺ release into the medium at 0- to 80-min time points was measured by scintillation counting. **B:** quantification of ⁸⁶Rb⁺ release at the 80-min time point from 3 experiments. Bars are means ± SE; n = 3 experiments. *P < 0.05.
K$^+$ efflux in enterocytes. As shown in Fig. 7A, MG-132 treatment led to p38 activation appearing at 45 min and steadily increased afterward, consistent with p38 involvement in the apoptotic response. Pretreatment with the specific p38 inhibitor SB202180 prevented proteolytic cleavage of procaspase 3 (Fig. 7B) and decreased the frequency of cells with active caspases (Fig. 7C) and frequency of cells with deenergized mitochondria (not shown) in cells treated with MG-132. Thus progression of MG-132-induced apoptosis to the caspase activation step critically depends on p38 activity. To examine whether p38 activation is required for apoptotic K$^+$ efflux, we measured the release of $^{86}$Rb$^+$ by apoptotic cells in the presence or absence of the p38 inhibitor. Figure 8 shows that SB202180 significantly decreased the efflux of $^{86}$Rb$^+$ after MG-132 treatment. This result points to p38 involvement in apoptotic activation of K$^+$ efflux in enterocytes.

**DISCUSSION**

In this study, we demonstrated that apoptosis in IEC-6 enterocytes is associated with the efflux of intracellular K$^+$ via K$^+$ channels. Because apoptosis does not occur, or is significantly attenuated after treatment with inhibitors of K$^+$ currents, K$^+$ efflux is an essential step in the apoptotic process. Our data indicate that K$^+$ efflux is required for all the manifestations of apoptosis that we examined, including DNA laddering, caspase activation, release of cytochrome c from mitochondria, and loss of mitochondrial membrane potential. Therefore, K$^+$ loss may be one of the earliest events in the apoptotic program. The requirement of K$^+$ efflux for apoptosis has been previously demonstrated in other cell types, namely, neurons (42), lymphocytes (7, 13, 16), cardiomyocytes (6), and smooth muscle cells (20). Our results demonstrate for the first time the critical requirement of K$^+$ efflux for apoptosis in enterocytes. We also show that in enterocytes, both apoptosis-specific K$^+$ efflux and apoptosis itself can be prevented by the specific pharmacological inhibitor of K$^+$ channels 48F10.

Our study shows that K$^+$ efflux is required for intrinsic, mitochondrial-type apoptosis. This type of programmed cell death occurs after injury by stresses such as DNA damage, protein denaturing, and oxidative stress. Treatment with MG-132 and peroxynitrite are examples of such stresses. The hallmark of intrinsic apoptosis is the loss of mitochondrial membrane potential with the subsequent release of cytochrome c and other apoptotic factors from mitochondria into the cytosol, and subsequent caspase activation. At this point, we do not know whether K$^+$ efflux also plays a role in extrinsic, receptor-mediated apoptosis in enterocytes. Because enterocytes may express death receptors such as CD95/FAS (33), it is possible that K$^+$ efflux is also involved in the extrinsic pathway of apoptosis in enterocytes, as was demonstrated for FAS-mediated apoptosis in lymphocytes (32).

The most intriguing question about the role of K$^+$ currents in apoptosis is how apoptotic stimuli evoke K$^+$ efflux. Our data demonstrate that the apoptotic surge of K$^+$ efflux in enterocytes depends on the p38 MAPK signaling pathway. p38 is strongly activated by MG-132 in IEC-6 cells. Other apoptosis-inducing stresses including DNA damage, oxidative stress, and osmotic shock also activate p38 in enterocytes (data not shown). p38 activity is required for the progression of apoptosis (as judged by caspase activation and deenergization of mitochondria) as well as for efficient apoptotic K$^+$ efflux. These data support the role of p38 in the apoptotic response to stresses and the associated K$^+$ efflux. Our finding of p38 mediating the apoptotic K$^+$ efflux in enterocytes agrees with the known role of p38 in apoptosis in various cell types including enterocytes (1, 34, 36). Moreover, p38 has been previously implicated in apoptotic K$^+$ current in neurons (24). It remains to be investigated whether activated p38 stimulates K$^+$ channel surface expression or affects biophysical properties of K$^+$ channels during apoptosis.

Before the mechanism of apoptotic K$^+$ current regulation can be tested, proapoptotic K$^+$ channels need to be identified. Our pharmacological data (Fig. 6) indirectly point to the voltage-gated channel Kv2.1 as a carrier of apoptotic K$^+$ efflux in IEC-6 cells. Indeed, apoptosis in IEC-6 cells is significantly attenuated in the presence of stromatoxin, which specifically blocks the Kv2.1 channel. However, we cannot exclude the contribution of other K$^+$ channels, particularly KvLQT1, which is strongly expressed in intestinal epithelia (38) and IEC-6 cells (Fig. 6A). Indeed, TEA, 48F10, and chromanol 293B, which inhibit KvLQT1 current, exert strong antiapoptotic effects in IEC-6 cells.

Our finding that the specific inhibitor of K$^+$ channels 48F10 cannot prevent apoptosis in enterocytes may have an important clinical implication. Intestinal disorders such as ulcerative colitis (3, 15, 44), coeliac disease (2, 22, 25), necrotizing enterocolitis (18, 28), and microvillus inclusion disease (10) are associated with increased levels of enterocyte apoptosis. Specific inhibitors of enterocyte K$^+$ channels may be useful in preventing or modulating gut barrier failure associated with these inflammatory conditions. 48F10 can be a prototype of such cytoprotective drugs. One of our future goals is to evaluate the efficacy of K$^+$ channel blockers in animal models of inflammatory bowel disease and necrotizing enterocolitis.

**ACKNOWLEDGMENTS**

We thank Dr. Colin Nichols and Dr. Ian Reynolds for the use of facilities in their laboratories, Dr. Robert Kass for KvLQT1-minK construct, and Dr. Elias Aizenman for helpful discussion.

**GRANTS**

This study was supported by National Institutes of Health Grants NS-O48089 (to E. Zaks-Makhina), A1-494-73-01 (to H. Ford), and HL-53312 (to E. S. Levitan) and by a start-up grant from the Research Advisory Committee Children’s Hospital of Pittsburgh (to A. Grishin).

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