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Potassium ion fluxes in corneal epithelial cells exposed to UVB

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ABSTRACT

The goal of this study was to investigate the efflux of K^+ from human corneal limbal epithelial cells (HCLE) exposed to ambient levels of UVB, which is known to cause apoptosis, and to examine the effect of K^+ channel blockers on loss of potassium induced by UVB. HCLE cells were exposed to 100–200 mJ/cm^2 UVB, followed by incubation in culture media with 5.5–100 mM K^+, BDS-1, Ba^{2+} or ouabain. To measure intracellular cations, cells were washed in 280 mM sucrose and lysed in DI water. K^+ and Na^+ levels in lysates were measured by ion chromatography. HCLE cells showed maximal loss of K^+_i 10 min after exposure to UVB and 5.5 mM K^+ media, with recovery of normal K^+ levels after 90 min. Treatment with 1 μM BDS-1 following UVB exposure reduced the loss of K^+ by HCLE cells. Exposure to 0.1–5 mM Ba^{2+} inhibited UVB-induced K^+ loss in a time and dose-dependent manner. These results confirm that blocking K^+ channels in HCLE cells exposed to UVB prevents efflux of K^+, confirming that UVB activates K^+ channels in these cells. Electrophysiology data show that K^+ channels remain highly active at least 90 min after UVB exposure. HCLE cells exposed to UVB and incubated in 0.01–1 μM ouabain did not recover from UVB-induced K^+ loss. These data suggest that the Na/K pump may act to restore $[K^+]_i$ to control levels in HCLE cells following UVB exposure and that the pump is not damaged by exposure to UVB. Incubation of HCLE cells exposed to UVB in medium with 25–100 mM K^+ media prevented K^+ efflux at extracellular concentrations as low as 25 mM (the concentration in tear fluid), maintaining control levels of K^+_i. In all experiments inward fluxes and intracellular Na^+ levels mirrored K^+ changes, albeit at the expected lower concentrations. The prevention of UVB-induced K_i^+ loss by 25 mM K_o^+ is consistent with the possible contribution of the relatively high K^+ concentration in tears to protection of the corneal epithelium from ambient UVB.

1. Introduction

Apoptotic pathways in cells are highly complex and can be activated by a large number of signals and intracellular pathways. It has been reported using in vitro systems and various cell types that an early event in the initiation of apoptosis is activation of K^+ channels with apparent loss of K^+ from the cell. As reviewed below, this has been observed after exposure of cells to compounds such as staurosporin and dexamethasone, as well as exposure to ultraviolet (UV) radiation. This effect of UV is of particular interest with regards to the corneal epithelium since the cornea is continually exposed to ambient outdoor UVB and UVA, and because damage to the corneal epithelium by exposure to UV is due, in part, to apoptosis (Podskochy et al., 2000; Lu et al., 2003; Wang et al., 2003; Shimmura et al., 2004). The activation of K^+ channels by UV in primary rabbit corneal epithelial cells, resulting in enhanced outward current and subsequent apoptosis, was demonstrated by Wang et al. (2003) and Lu et al. (2003). In their experiments cells were exposed to UVC to which corneas are not normally exposed since UVC is filtered by the atmosphere. We subsequently reported that UVB at doses relevant to ambient outdoor exposure also activates K^+ channels and causes apoptosis of human corneal limbal epithelial (HCLE) cells in culture (Singleton et al., 2009) and that blocking the K^+ channels after UVB exposure inhibits apoptosis (Ubels et al., 2010).

If initiation of apoptosis is due, at least in part, to loss of K^+ from cells, then incubation of cells in elevated extracellular $[K^+]$ after exposure to conditions that cause apoptosis should inhibit apoptotic mechanisms. Bortner et al. (1997) and Hughes et al. (1997) reported that chemically induced apoptosis of lymphocytes can be prevented by incubation of the cells in isosmotic

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medium with 103 mM K^+. This concentration is near intracellular levels, eliminating the concentration gradient and preventing loss of K^+ from the cells. Yu et al. (1997) reported that 25 mM K^+_o, which is well above normal levels in culture medium (about 5 mM), reduced outward K^+ current and inhibited neuronal cell apoptosis in vitro after serum deprivation or staurosporin exposure. They suggested that this was due to reduction of K^+ loss from the cells in spite of channel activation. These observations are intriguing with respect to the corneal epithelium, which is continually bathed in tears that have a K^+ concentration of about 25 mM, significantly higher than levels in extracellular fluid and other exocrine secretions (Atta et al., 1975; Botelho and Martinez, 1973; Rismondo et al., 1989). Previously, we proposed that the relatively high $[K^+]$ in tears reduces the outward gradient for K^+ loss, inhibiting apoptotic pathways and preventing UVB damage to the corneal epithelium. In support of this hypothesis we reported that incubation of HCLE cells in isosmotic medium with 25–100 mM K^+ for 6 h after exposure of the cells to 100–200 mJ/cm^2 UVB reduced DNA fragmentation and inhibited activation of caspase-3 and caspase-8 (Singleton et al., 2009).

The purposes of the present study were to further investigate effects of UVB on the magnitude and time course of intracellular K^+ (K_i^+) loss from HCLE cells and to measure the effect of blocking K^+ channels on UVB-induced loss of K_i^+. Most importantly, we show that incubation of the cells in medium with a $[K^+]$ as low as 25 mM after exposure to 150 mJ/cm^2 UVB maintains $[K^+]_i$ at levels consistent with inhibition of UVB-induced apoptosis. Since it has been suggested that Na^+ fluxes resulting in increased $[Na^+]_i$ are also involved in apoptosis (Bortner and Cidlowski, 2003, 2007; Bortner et al., 2008; Arrebola et al., 2005), UVB-induced changes in Na^+ levels were also measured.

2. Materials and methods

2.1. Cell culture

Human corneal limbal epithelial cells (HCLE) were maintained as monolayer cultures in Keratinocyte-Serum Free Medium (KSFM, Invitrogen, Carlsbad, CA), as previously described (Gipson et al., 2003; Singleton et al., 2009).

2.2. UVB exposure

Cells were grown to confluence in the four corner wells of six-well plates in KSFM with the standard 5.5 mM K^+. The medium was changed to Hank's Balanced Salt Solution (HBSS) without phenol red (Invitrogen), and the cells were exposed to 302 nm UVB at 100–200 mJ/cm^2 using an Ultraviolet Products model UVM-57 lamp (UVP, Upland, CA), which delivers an equal dose of UVB to the cells in each well, as described by Singleton et al. (2009). UVB intensity was measured with a Solarmeter Model 6.2 (Solartech, Inc., Harrison Twp., MI). Control cells were subjected to the same medium changes as treated cells, without the exposure to UVB.

2.3. Measurement of intracellular cation content by ion chromatography

After exposure to UVB, the HBSS was immediately aspirated and the cells were incubated in KSFM with varying K^+ concentrations or pharmacologic agents for time periods ranging from 10 to 90 min, as explained in the Results section. The high K^+ medium was prepared by mixing normal medium with custom-made KSFM containing 100 mM K^+ and reduced Na^+ to maintain osmolarity (Invitrogen).

The protocol used for measurement of intracellular cations was based on a method described by Friis et al. (2005), with major modifications. At the end of the incubation period the medium was aspirated, and the cells were washed twice with 2 ml of 280 mM sucrose in DI water to remove extracellular electrolytes. The cells were lysed by adding 500 μL of deionized water to each well and placing the plate in a −80 °C freezer for 5 min, followed by rapid thawing in a 37 °C incubator. The cell debris was scraped from the well using a cell scraper, and the lysate with the cell debris was transferred to a 1.5 ml microcentrifuge tube. After centrifugation at 10,000 × g for 3 min, 50 μL of supernatant was retained for use in the Bio-Rad protein assay (Bio-Rad, Hercules, CA). The remaining 350–400 μL of supernatant was transferred to an Amicon Ultra-0.5 Centrifugal Filter Device and centrifuged for 30 min at 14,000 × g to remove proteins greater than 3 kD, since larger proteins would interfere with the function of the ion chromatography column.

Cations in the lysates, all of which were of intracellular origin, were measured by ion chromatography using a Dionex DX500 ion chromatograph with a Dionex CS-12 cation column and ED50 electrochemical detector (Dionex, Sunnyvale, CA). The mobile phase was 11 mM H_2SO_4 in degassed, deionized water. K^+ and Na^+ concentrations in the samples were calculated based on a cation standard curve and PeakNet software integral to the ion chromatograph. Ion concentrations in the cell lysates were expressed as μg/mg protein.

2.4. Patch-clamp recording

HCLE cells were plated in a recording chamber at low density in bath solution containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES and 10 glucose at pH 7.4. Whole-cell voltage-clamp current recordings were made using patch pipettes (resistance 2–5 MΩ) filled with a recording solution containing (in mM) 145 K-methanesulfonate, 2.5 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES and 0.25 mg/ml amphotericin-B (Sigma, St. Louis, MO) at pH 7.3, as previously described (Singleton et al., 2009; Ubels et al., 2010). To determine the time course of UVB-induced K^+ channel activation, from a holding potential of −80 mV command voltage steps were given at 10 mV, 250 ms intervals to +100 mV. After 3 control protocols cells were exposed to 80 mJ/cm^2 UVB and the recording protocol was repeated immediately after exposure and at intervals of several minutes for as long a cell access could be maintained. Data were analyzed from cells in which access was maintained for 45–90 min. Although most ion chromatography experiments in this study used a 150 mJ/cm^2 UVB dose, patch recordings were conducted using a dose of 80 mJ/cm^2 because, as previous reported (Singleton et al., 2009), the higher dose causes unacceptably high access resistance, preventing long term recording.

2.5. Statistical analysis

Ion chromatography data were analyzed statistically by analysis of variance and the Student–Newman–Keuls test. All data are presented as mean ± standard deviation, and differences were judged to be statistically significant at $p \leq 0.05$.

3. Results

3.1. Effect of UVB on intracellular K^+ and Na^+ levels of HCLE cells

Initial experiments were conducted by exposing HCLE cells to 150 mJ/cm^2 UVB and incubating the cells in KSFM for 2–6 h, a time range chosen based on the response of apoptotic signaling pathways to UVB in our previous studies (Singleton et al., 2009; Ubels et al., 2010). No effect on $[K^+]_i$ as compared to untreated controls