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Noninvasive Measurement of Potassium Efflux as an Early Indicator of Cell Death in Mouse Embryos[1]

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ABSTRACT

Programmed cell death (apoptosis) occurs in nearly all cell types examined, including mammalian oocytes and embryos, where it may underlie some forms of infertility in humans. Although the molecular machinery participating in apoptosis have been intensely investigated, the accompanying physiological changes have not received similar attention. In this study, a novel electrophysiology technique has been employed to monitor real-time perturbations in the physiology of mouse embryos undergoing apoptosis evoked by hydrogen peroxide, diamide, and staurosporine. Despite differences in their mode of action, these agents evoked a similar early change in cellular physiology; namely, a pronounced, transient, potassium efflux through tetraethylammonium-sensitive potassium channels accompanied by cell shrinkage. Mouse zygotes exposed to 200 μM H_2O_2 exhibited potassium efflux that elevated the potassium concentration of the media surrounding embryos by 1.4 ± 0.1 μM. Pretreatment with tetraethylammonium inhibited this increase (0.2 ± 0.1 μM). Our results indicate that potassium efflux through potassium channels and concurrent cell shrinkage are early indicators of cell death in embryos and that noninvasive measurements of potassium pathophysiology may identify embryos undergoing cell death prior to the manifestation of other morphological or molecular hallmarks of cell death.

apoptosis, developmental biology, signal transduction

INTRODUCTION

Similar to somatic cells, dying mammalian oocytes and embryos exhibit characteristic morphological features, including membrane blebbing, cytoplasmic fragmentation, and cell shrinkage [1–8]. Indeed, these cytological features are used to select embryos for transfer during assisted reproduction in women and domestic species [9–11]; however, the physiological mechanisms underlying these morphological modifications have not been extensively investigated. We therefore have begun to investigate the physiological transformation accompanying cell death in embryos. Initially, we focused on the biophysical mechanisms underlying cell shrinkage because cell shrinkage is a distinctive feature of apoptotic or programmed cell death and is in direct contrast to cellular swelling, which occurs during necrosis or insult-induced cell death [12, 13].

Cell volume regulation is accomplished through ion homeostasis across the plasma membrane. Potassium, the most abundant intracellular ion, plays a major role in cell volume regulation [14–17], and alterations in potassium homeostasis are associated with disruptions in cell function and cell death [12, 16, 18–22]. Somatic cells shrink by the movement of potassium ions down their electrochemical gradient, through potassium channels in the plasma membrane, and out of the cell [12, 16, 18, 20]. This exodus of potassium ions is followed by compensatory osmotic water movement that results in cell shrinkage. In embryos, potassium ions have previously been shown to participate in cell volume regulation [14, 16], and cell death in embryos is associated with cell shrinkage [2, 6, 7, 23]. Therefore, altered potassium homeostasis may provide a physiological indicator of the early stages of embryonic cell death.

Ion movements across the plasma membrane of single cells can be monitored noninvasively by employing the self-referencing ion-selective technique [24, 25]. The exquisite sensitivity of this physiological method arises by moving an electrode between two positions 10 μm apart. One pole of the oscillation measures the physiological signal near the cell while the other measures the physiological signal at a position remote from the cell. By obtaining the difference between the measurements at these two sites, the contribution of electronic drift is reduced, and the resultant signal obtained is proportional to the difference in the concentration of the particular ion species at these two positions. This technique has been used to noninvasively monitor physiological parameters from a variety of cell types [24, 25], and recently was employed to monitor the physiology of oocytes [26, 27] and embryos [28, 29]. In addition, we have shown that the self-referencing technique does not compromise the subsequent development of embryos (unpublished observations). Therefore, the self-referencing technique is uniquely suited to measure potassium homeostasis from oocytes and preimplantation embryos as they die.

For this study, we employed a potassium-selective electrode in self-referencing mode to characterize, in real-time, potassium efflux from individual dying embryos evoked by three pharmacological agents, hydrogen peroxide (H_2O_2) [30–32], diamide [23], and staurosporine [7, 2, 33]. Although these agents act on different levels of the apoptotic pathway (H_2O_2 and diamide evoke oxidative stress whereas staurosporine inhibits protein kinases), we report here that all three evoked a common, early change in cellular physiology; namely, potassium efflux through tetraethylammonium-sensitive potassium channels and cell shrinkage. These findings indicate that potassium efflux is a common early event of apoptosis in embryos and that the self-referencing electrode technique can be used to noninvasively study the real-time pathophysiology of cells undergoing cell

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death prior to the manifestation of other morphological or molecular hallmarks of cell death.

MATERIALS AND METHODS

The self-referencing system used to monitor potassium gradients around embryos was identical to that previously described [24, 25, 27, 29]. Briefly, the system was composed of a Zeiss Axiovert 100 TV inverted microscope (Carl Zeiss, Inc., Thernwood, NY) with a modified stage plate to which computer-controlled micromanipulators were affixed. The microscope rested on a Kinetic Systems Vibraplane vibration-resistant table (Boston, MA) enclosed in a stainless steel insulated chamber. The temperature of the chamber and its contents, including the microscope and manipulators, was adjusted to 37°C. Physiological measurements were conducted in Hepes-buffered potassium simplex-optimized medium (HKSOM) containing reduced $NaHCO_3$ (4 mM) and elevated Hepes (14 mM). Recordings were obtained from embryos in plastic Petri dishes with a cover glass bottom (MatTek Corp., Ashland, MA), which facilitated embryo positional stabilization.

Potassium-selective electrodes (tip diameter of 3–5 μm) were fabricated using K+-ionophore I-cocktail B (Fluka, Milwaukee, WI) and back-filled with 100 mM KCl as previously described [34]. All electrodes were calibrated and confirmed to be Nernstian prior to use. A silver/silver chloride reference electrode completed the circuit in solution by way of a 3 mol L^{-1} NaCl/3% agar bridge. During recording, the electrode was oscillated in a square wave parallel to the electrode axis over a distance of 10 μm with a frequency of 0.3 Hz. The near position of this oscillation was 1.5–2.5 μm from the zona pellucida (ZP) or plasma membrane, in cases where the ZP was removed by brief pronase digestion. Data acquisition and manipulation were performed as described previously [24, 25]. The hardware and software controlling electrode movements, signal amplification, and data acquisition were designed and constructed by the BioCurrents Research Center at the Marine Biological Laboratory, Woods Hole, MA (www.mbl.edu/BioCurrents). Data from individual embryos were interpolated and averaged using programs employing the interpolation function of MatLab software (MatLab Corp., Cambridge, MA). Interpolation between data points gathered from any individual embryo allowed calculation of the average and errors from a group of zygotes from which data were gathered at different times relative to the pharmacological treatment.

Digital images of embryos were captured periodically throughout the experiment using a Cohu analog video camera (Cambridge Research Instruments, Cambridge, MA) and a personal computer running Metamorph software (Universal Imaging Corp., West Chester, PA). The morphometric features of zygotes were analyzed from these images using Metamorph.

Female B6C3F1 mice (6 wk old) were purchased from Charles River (Boston, MA) and subjected to a 14L:10D cycle for at least 1 wk before use. Animals were cared for according to procedures approved by the Marine Biological Laboratory and Women and Infants Hospital Animal Care Committees. Male B6C3F1 mice of proven fertility were used for mating. Female mice were superovulated by i.p. injection of 7.5 IU eCG (Calbiochem, La Jolla, CA) followed 46–48 h later by injection of 7.5 IU human chorionic gonadotropin (hCG), and mated individually. Females exhibiting mating plugs were selected the next morning and killed by cervical dislocation at 22–23 h after hCG injection. Zygotes (~30/animal) enclosed in cumulus masses were released from the ampullae into HKSOM supplemented with 0.03% hyaluronidase. Cumulus cells were gently removed from zygotes by pipetting. Cumulus-free zygotes were washed in Hepes-buffered KSOM three times and then in pre-equilibrated modified KSOM three times. Modified KSOM used for in vitro culture was supplemented with nonessential amino acids (1 ml of 100× stock/100 ml media) and 2.5 mM Hepes. Embryos were pooled, randomly distributed, and cultured in 50-μl droplets under mineral oil at 37°C in a humidified atmosphere of 7% CO_2 in air. These embryo harvest and culture procedures sustained development of control 1-cell zygotes to blastocysts (>90%) and blastocysts resulting from 4 days of in vitro culture developed to viable offspring when transferred to pseudopregnant female mice. For most experiments, the ZP was mechanically removed following mild treatment with pronase. No difference in physiological signals was observed if the embryos were examined immediately following ZP removal or after a 0.5–2.5 h incubation/recovery period. Zona-free embryos cleaved in a manner similar to zona-intact sibling embryos. All reagents were purchased from Sigma Chemical Company (St. Louis, MO), unless stated otherwise.

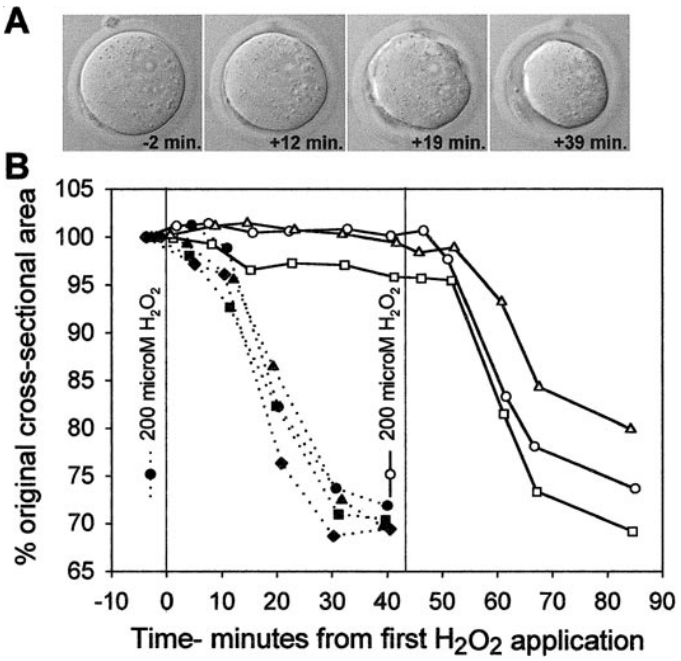

FIG. 1. Cell shrinkage induced by H_2O_2. Zygotes treated with 200 μM H_2O_2 underwent shrinkage during the first 40 min of exposure. **A**) Images of a shrinking zygote taken at various times before (−2 min) and after (+n min) treatment with H_2O_2 (treatment at 0 min). **B**) Shrinkage of zygotes coincided with H_2O_2 treatment. Embryos began to shrink immediately upon H_2O_2 treatment (filled symbols) whereas exposure of embryos to the imaging conditions did not evoke shrinkage until H_2O_2 was added (open circles). Data were gathered from images obtained at an equatorial focal plane and presented as the cross-sectional area of zygotes relative to their original cross-sectional area in the first image (100%). Each line and associated symbols represents an individual zygote.

RESULTS

Cell Shrinkage Induced by H_2O_2

Exposure of 1-cell mouse zygotes to 200 μM H_2O_2 resulted in cell shrinkage and membrane blebbing characteristic of apoptotic cell death (Fig. 1A) [30]. A dramatic de-

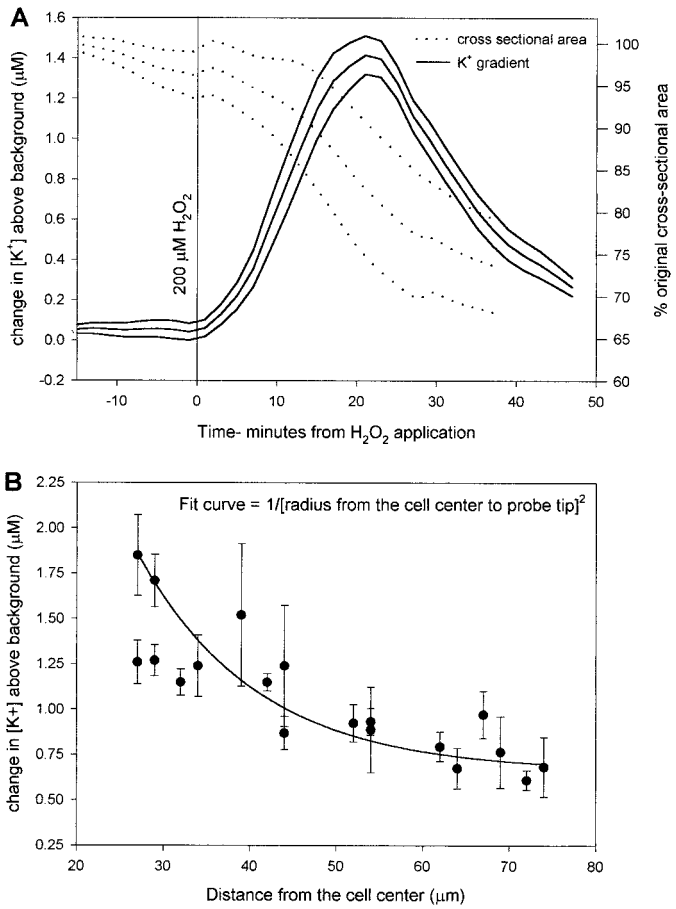

FIG. 2. Hydrogen peroxide induces shrinkage and potassium efflux from mouse zygotes. **A**) Treatment of zygotes with 200 μM H_2O_2 evoked cell shrinkage (dotted lines) and an increase in the potassium concentration of the media near embryos (solid lines). Potassium concentration data are presented as the increase in the potassium concentration of the media near embryos above that of the bulk media (2.5 mM = 0). In each case, the middle line represents the interpolated mean value at a time relative to that of H_2O_2 application (0 min), while the outer two lines represent the interpolated standard error of the mean from the 21 embryos tested. Cell shrinkage data were gathered from images obtained at an equatorial focal plane and presented as the mean cross-sectional area of zygotes relative to their original cross-sectional area in the first image (100%). At its peak, the potassium concentration near embryos was approximately 1.5 μM above that of the bulk media and coincided with a rapid rate of cell shrinkage. **B**) The gradient of potassium concentration around H_2O_2-treated zygotes decreased with increasing distance from the embryo at a rate of $1/r^2$, where r is the distance from the center of the cell to the probe tip. The line fits the equation $1/r^2$ for these distances. Measurements were taken at various distances from the surface of four H_2O_2-treated zygotes and suggest that H_2O_2 induced potassium efflux across the zygote plasma membrane. Error bars represent the standard deviation in the measurement at a particular distance from the center of an individual embryo.

crease in cell volume was apparent in the cross-sectional area as observed in images obtained at an equatorial focal plane (Fig. 1). Zygotes began to shrink within 5 min after H_2O_2 treatment and continued shrinking during the 10–30 min following H_2O_2 exposure. Zygotes shrunk at a maximum rate of 1.19% from the original cross-sectional area per min (Fig. 1B). The cross-sectional area of zygotes 40 min after exposure to H_2O_2 was only 68% of the original area and coincided with a decrease in diameter of more than 15%, and of approximately 40% in volume (assuming a spherical shape). This rapid shrinkage was an immediate response to H_2O_2 exposure and was not a result of exposure to the imaging conditions. Embryos maintained their original size when imaged over a period of 40 min and decreased in cross-sectional area only when treated with H_2O_2 (Fig 1B).

Potassium Efflux Induced by H_2O_2

Concurrent with cell shrinkage, H_2O_2 induced potassium efflux from zygotes (Fig. 2). Initial recordings using the self-referencing electrode technique demonstrated that H_2O_2 evoked potassium efflux from zona-intact embryos. However, these observations were quantitatively variable because this technique required that the geometric relationship between the embryo plasma membrane and the electrode tip remain constant. Yet, as the embryos shrunk, the plasma membrane retracted from the inner surface of the ZP and, thereby, altered this geometric relationship. Removal of the ZP allowed repositioning of the electrode as the embryos shrunk, and all of the data we present here were obtained from embryos from which the ZP was removed, although similar results were obtained from zona-intact embryos.

Treatment of mouse zygotes with 200 μM H_2O_2 induced a rapid increase in the potassium concentration of the media near individual embryos (Fig. 2). Initially, potassium concentrations near embryos were indistinguishable from those of the bulk media (2.5 mM; Fig. 2A). However, 20 min following exposure to H_2O_2, potassium concentrations around embryos had risen to a peak of 1.4 ± 0.1 μM above that of the bulk media and subsequently slowly decreased to background levels (Fig. 2). This potassium formed a gradient near each embryo such that the concentration of potassium decreased with increasing distance from the embryos (Fig. 2B). This potassium gradient extended away from the embryo surface, tens of micrometers into the media with a relationship of 1/[the radius from the center of the cell to the probe tip]^2. This relationship, $1/r^2$, is indicative of a gradient around a potassium source and suggests that H_2O_2 induced an efflux of potassium down its electrochemical gradient, across the embryo plasma membrane, and into the media. The potassium gradient we observed around individual embryos corresponds to a peak potassium efflux of 0.3 pmol of potassium/embryo/min and raised the potassium concentration of the media near embryos by only 1/2000th over that of the bulk media (from 2.5 mM to 2.5014 mM), yet was unequivocally detectable using the self-referencing electrode technique.

Measurements of the cross-sectional area of zygotes simultaneously with potassium efflux determinations indicated that potassium efflux began slightly prior to cell shrinkage and that peak potassium efflux correlated temporally with the maximum rate of cell shrinkage. On average, the potassium efflux began 2.8 ± 3.1 min after H_2O_2 exposure and approximately 2.3 ± 2.1 min prior to the onset of cell shrinkage (Fig. 2B). The peak potassium efflux occurred 21.2 ± 2.4 min following H_2O_2 treatment (Table 1) and simultaneously with the greatest rate of embryo shrinkage (1.2% decrease in embryo cross-sectional area per min; Fig. 2A). The potassium efflux continued coincident with shrinkage and promptly declined upon cessation of shrinkage (Fig. 2A). Twenty-four hours after exposure to H_2O_2 for 15 min, rinsing, and subsequent culture, no potassium efflux was evident (the potassium concentration near embryos was 0.08 ± 0.13 μM above media concentrations and indistinguishable from background levels).

TABLE 1. Summary of potassium efflux and cell shrinkage of mouse zygotes induced by apoptotic agents.

Agent*	Maximum [K^+] above background (μM)†	Time of maximum [K^+] (min after drug application)	Rate of shrinkage‡ (% change in cross-sectional area/min)
H_2O_2	1.42 ± 0.10	20–22	1.2
TEA + H_2O_2	0.23 ± 0.08	18–23	0.4
NaCl + H_2O_2	1.25 ± 0.27	21–26	0.7
Diamide	0.69 ± 0.17	15–30	0.5
Staurosporine	0.62 ± 0.23	11–14	0.4

* Numbers of zygotes treated are as follows: 21 H_2O_2, 21 TEA + H_2O_2, 11 NaCl + H_2O_2, 11 diamide, and 13 staurosporine.
† Average and SEM of the peak [K^+] concentration.
‡ Rate of shrinkage when the [K^+] above background was maximum.

The Potassium Channel Blocker, Tetraethylammonium Chloride, Inhibits Potassium Efflux and Cell Shrinkage

Hydrogen peroxide-induced potassium efflux could arise from potassium movement across the plasma membrane either through potassium channels or nonspecifically through loss of plasma membrane integrity. Tetraethylammonium chloride (TEA) blocks of a variety of plasma membrane potassium channels [35]. Embryos pretreated with TEA (75 mM) and subsequently exposed to 200 μM H_2O_2 in the presence of TEA exhibited decreased potassium efflux and reduced shrinkage rate (Fig. 3; Table 1). The potassium concentration near zygotes induced by H_2O_2 in the presence of TEA was only 18% that of control embryos treated with NaCl (35 mM) and H_2O_2 in a similar manner. Sodium chloride (35 mM) was employed as a control for the change in osmolarity evoked by 75 mM TEA (Fig. 3).

The concentration of TEA employed, 75 mM, is similar to that used to block potassium channels in neurons [35]; however, this concentration also raised the osmolarity of the physiological media from 240 mOsm to 290 mOsm. To determine whether the inhibition of potassium efflux and cell shrinkage by 75 mM TEA resulted solely from the change in osmolarity, rather than a pharmacological block of potassium channels, we treated embryos with 35 mM NaCl in a similar manner as was done for TEA. Although 35 mM NaCl mimicked the osmolarity change established by TEA (240–290 mOsm), it did not affect H_2O_2-induced potassium efflux and cell shrinkage (Fig. 3; Table 1). The peak potassium concentration near H_2O_2-treated zygotes pretreated with NaCl was 1.25 ± 0.27 μM (Fig. 3) and the general kinetics of the potassium efflux were similar to that of control embryos treated with H_2O_2 alone (Fig. 1). The rate of cell shrinkage observed in H_2O_2-treated zygotes pretreated with NaCl, however, was slightly less than that of control zygotes (Table 1). It is interesting that the cell shrinkage osmotically induced by NaCl alone during the pretreatment period was not accompanied by potassium efflux (Fig. 3).

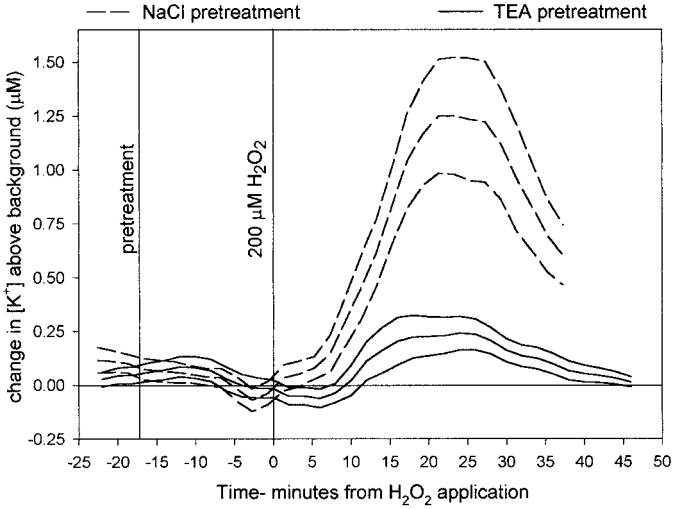

FIG. 3. TEA inhibits H_2O_2-induced potassium efflux. Zygotes pretreated with TEA (75 mM) and exposed to H_2O_2 (200 μM) in the presence of TEA failed to exhibit potassium efflux as seen as only a modest increase in the potassium concentration of the media near embryos above that of the bulk media (2.5 mM = 0). Control zygotes pretreated with NaCl (35 mM) and exposed to H_2O_2 (200 μM) in the presence of NaCl exhibited potassium efflux with kinetics similar to untreated zygotes (compare with Fig. 2A). Sodium chloride was used to control for the osmolarity change (240–290 mOsm) evoked by TEA. In each case, the middle line represents the interpolated mean value at a time relative to that of the H_2O_2 application (0 min), whereas the outer two lines represent the interpolated SEM from the embryos tested (n = 21 TEA + H_2O_2, 11 NaCl + H_2O_2).

Diamide and Staurosporine Induce Cell Shrinkage and Potassium Efflux

We sought to determine whether cell shrinkage and potassium efflux were a general phenomenon of pharmacologically induced cell death in mammalian embryos. Treatment of zygotes with 50 μM diamide induced a pronounced potassium efflux accompanied by cell shrinkage (Fig. 4A). Diamide-induced an increase in the potassium concentration around embryos that began 1–3 min after diamide exposure and approximately 3 min prior to the onset of cell shrinkage (Fig. 4A). By 15 min after exposure of diamide, the potassium concentration around embryos reached a peak of 0.69 ± 0.17 μM above the bulk media, and the potassium efflux continued for approximately 15 min before returning to background levels (Fig. 4A, Table 1). Concomitant cell shrinkage was induced by diamide and occurred at a rate of change from the original cross-sectional area of 0.53% per min (Table 1). Staurosporine (10 μM) also induced an elevation in the concentration of potassium (0.62 ± 0.23 μM) in the media near embryos and coincident cell shrinkage at a rate of 0.42% change in cross-sectional area per min (Fig. 4B, Table 1). The amplitude and kinetics of the increase in potassium concentration near embryos evoked by diamide and staurosporine are different from those evoked by H_2O_2 (Figs. 2 and 3), but most likely reflect differences in the effective dose of the compounds employed.

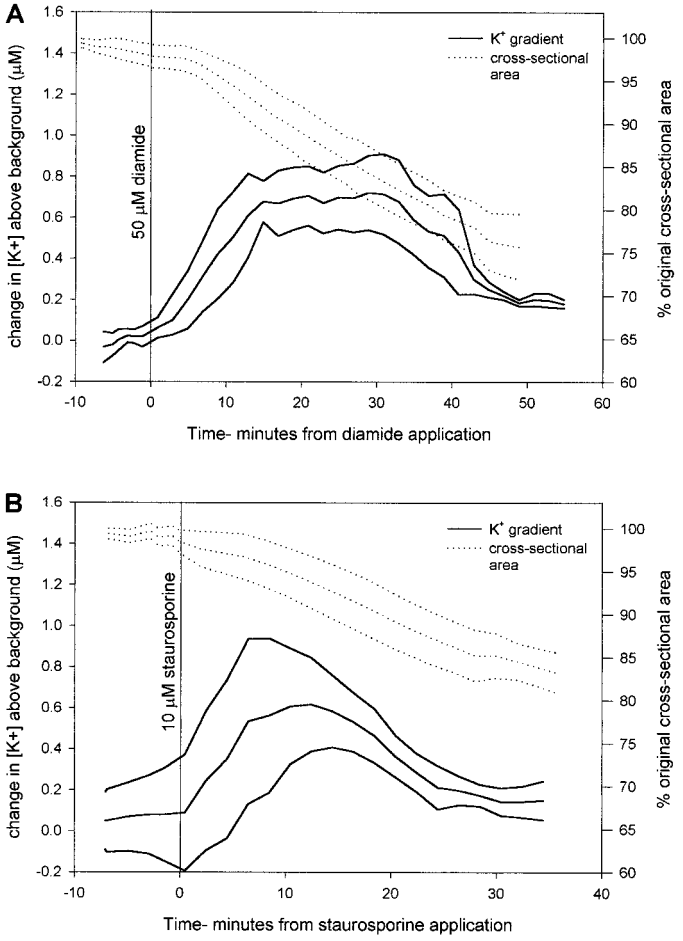

FIG. 4. Diamide and staurosporine evoke cell shrinkage and potassium efflux. Treatment of zygotes with 50 μM diamide (**A**) or 10 μM staurosporine (**B**) evoked cell shrinkage (dotted lines) and potassium efflux as seen as an increase in the potassium concentration of the media near embryos (solid lines) above that of the bulk media (2.5 mM = 0). In each case, the middle line represents the interpolated mean value at a time relative to that of the H_2O_2 application (0 min), whereas the outer two lines represent the interpolated SEM from the embryos tested. Diamide evoked a moderate elevation in the concentration of potassium near embryos coincident with gradual cell shrinkage. Immediately following staurosporine treatment, a mild increase in the potassium concentration near zygotes was observed coincident with a slow rate of cell shrinkage (n = 11 diamide, 13 staurosporine).

DISCUSSION

We present here the first real-time measurements of physiological perturbations associated with the death of individual mammalian embryos. Mouse zygotes, pharmacologically treated with agents known to induce cell death, exhibited potassium efflux through TEA-sensitive potassium channels and concomitant cell shrinkage during the first 40 min of exposure. This cell death-associated potassium efflux occurs prior to the manifestation of other hallmarks of apoptotic cell death, including changes in mitochondria membrane potential, caspase activation, cytochrome C release, annexin-V labeling, loss of plasma membrane integrity, and DNA fragmentation [7, 23, 30, 33, 36]. Potassium efflux and cell shrinkage were early features common to cell death induced by three agents that act at very different levels within the apoptotic pathway. Hydrogen peroxide is a general oxidizing agent, diamide oxidizes glutathione-rich mitochondrial membranes, and staurosporine is a broad-spectrum kinase inhibitor. Therefore, altered potassium homeostasis can serve as an early prognosticator that an embryo is undergoing cell death, independent of the root cause of death. To our knowledge, the self-referencing electrode technique provides the only noninvasive physiological monitor of cell death currently applicable to single oocytes or embryos, and may prove valuable as a diagnostic tool for identifying developmentally incompetent embryos.

Potassium efflux has been shown to be one of the earliest events during apoptosis of somatic cells and is presumed to be necessary for activation of the apoptotic biochemical cascade [18–22, 37]. Intracellular enzymes necessary for the execution of apoptosis (caspases) are inhibited by the elevated concentration of potassium within healthy cells. During apoptosis, potassium concentrations decrease to levels that activate these enzymes [18, 19]. The most likely mechanism by which intracellular potassium concentrations decrease during apoptosis is potassium efflux into the surrounding media. Potassium may exit the cell by passing through either potassium channels in the plasma membrane or nonspecifically through a loss of plasma membrane integrity, which is common in late stages of apoptosis and early stages of necrosis. The potassium efflux we observed from zygotes treated with apoptotic agents most likely results from potassium movement through potassium channels. While H_2O_2 and diamide both evoke a loss of membrane integrity (e.g., propidium iodide-permeable), nonspecific membrane permeation occurs much later in the progression of apoptosis [26, 30] than does the potassium efflux documented here. Furthermore, the potassium channel blocker, TEA [35], inhibited apoptosis-associated potassium efflux and cell shrinkage. Likewise, some apoptotic agents, such as H_2O_2, can act directly on potassium channels [21, 22, 38, 39]. These results together indicate that during apoptosis in mouse zygotes, potassium exits the cell through potassium channels in a manner consistent with that occurring during apoptosis in somatic cells [18–22].

Efflux of potassium through potassium channels most likely underlies the cell volume decrease we observed in zygotes undergoing apoptosis. In somatic cells, potassium efflux occurs coincident with cell shrinkage [17–22]. In zygotes, potassium efflux has been implicated in volume regulation following hypotonic swelling [14]. It is likely that the potassium efflux we document here underlies the cell shrinkage observed during apoptotic death because three apoptotic agents induced potassium efflux slightly prior to the onset of cell shrinkage, the peak potassium efflux occurred coincident with the greatest rate of cell shrinkage, and termination of cell shrinkage was associated with a decline in potassium efflux. Moreover, the magnitude of potassium efflux exponentially correlated with the rate of shrinkage ($r = 0.9$; peak rate of shrinkage = $0.24 \cdot \exp^{(1.03 \cdot \text{peak change in [K+] above background})}$). For example, H_2O_2 evoked a large potassium efflux and rapid rate of shrinkage, whereas diamide and staurosporine evoked more moderate potassium efflux and slower rates of shrinkage. Inhibiting potassium efflux from zygotes with a potassium channel blocker also reduced the rate of cell shrinkage. Therefore, it is likely that apoptotic agents induce an exodus of potassium ions that is followed by compensatory osmotic water movement, resulting in cell shrinkage. It is interesting that shrinkage induced by hyperosmotic conditions (35 mM NaCl) was not associated with potassium efflux, suggesting that water can move out of the cell in a potassium-independent fashion, and that the water movement observed during apoptotic cell death may not occur by the loss of hydrated potassium, but rather water move-

ment associated with changes in the osmotic turgor within the cell that result from the redistribution of the ionic osmolite, potassium.

Although the broad-spectrum potassium-channel blocker, TEA [35], is capable of inhibiting apoptosis-associated potassium efflux and cell shrinkage, it was unable to rescue embryo development from H_2O_2 treatment. Embryos pretreated with TEA and exposed to 200 μM H_2O_2 (15 min) in the presence of TEA failed to cleave or develop (data not shown). Complete inhibition of potassium efflux was not possible with TEA and the residual potassium efflux induced by H_2O_2 in the presence of TEA might promote cell death. Alternatively, H_2O_2 may impair several parallel pathways within the cell and TEA acts upon only two, potassium efflux and cell shrinkage.

Mammalian oocytes and embryos, including those of humans, exhibit morphological hallmarks of apoptotic cell death; notably, cell shrinkage [1–8]. We have found that there is no measurable net potassium efflux from healthy embryos, but that apoptotic stimuli induce potassium efflux and cell shrinkage. Although we observed potassium efflux over a defined brief period (~1 h) during the initial stages of pharmacologically induced cell death, we do not yet know whether zygotes dying naturally also exhibit altered potassium homeostasis or over what time course. Our findings demonstrate that potassium efflux is an early indicator of pharmacologically induced cell death in embryos, and that noninvasive measurements of potassium efflux can identify embryos undergoing cell death prior to the manifestation of other morphological or molecular hallmarks.

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