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Prostaglandin D_2 synthase induces apoptosis in pig kidney LLC-PK1 cells

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Background. Prostaglandin D_2 synthase (PGD_2S), a unique member of the lipocalin family, is found at elevated levels in the serum of patients with renal impairment and has recently been implicated as a new biochemical marker of renal insufficiency. The aim of this study was to investigate the apoptotic effects of PGD_2S on a pig kidney epithelial cell line (LLC-PK1) and to investigate the effects of prostaglandins and growth factors on this process.

Methods. Apoptosis was detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL), annexin V staining, and electron microscopy.

Results. A four- to fivefold increase in apoptosis was observed in PGD_2S-treated cells as compared with controls and the apoptosis appeared to act via caspase-3. A cyclooxygenase-2 inhibitor, anti-PGD_2S antibody, and selenium all significantly inhibited the apoptosis induced by PGD_2S; however, none had any effect on the apoptosis induced by the known apoptotic inducer camptothecin. Furthermore, prostaglandins E_1 and E_2, known to induce mitogen-activated protein (MAP) kinase phosphorylation and exhibit cytoprotective effects, both inhibited PGD_2S-induced apoptosis, while prostaglandin H_2 had no significant effect. Growth factors such as insulin, insulin-like growth factor-1, and platelet-derived growth factor also decreased PGD_2S-induced apoptosis. In addition, PGD_2S isolated from human serum seemed slightly more effective at inducing apoptosis than recombinantly expressed protein.

Conclusions. We report on the induction of apoptosis by PGD_2S in LLC-PK1 pig kidney epithelial cells, and speculate that the accumulation of PGD_2S in the serum of kidney failure patients may further exacerbate renal problems and is most likely regulated by other prostaglandins and growth factors.

Lipocalin-type prostaglandin D_2 synthase (PGD_2S), also known as β-trace protein, is responsible for the conversion of prostaglandin H_2 (PGH_2) into prostaglandin D_2 (PGD_2). PGD_2, reported to induce sleep, inhibit platelet aggregation, inhibit nitric oxide release, induce vasodilation, and act as an allergic and inflammatory lipid mediator [1], is the precursor of 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 (15-$dPGJ_2$), a natural peroxisome proliferator-activated receptor-γ (PPARγ) ligand known to cause apoptosis [2]. Depending on the glycosylation status, PGD_2S migrates anywhere from 20 to 29 kD on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The enzyme, originally discovered as a major protein of human cerebrospinal fluid, has a dual function since it also acts as a lipophilic ligand-binding protein, binding and transporting retinoids, thyroids and bile pigments [1]. Recently, PGD_2S levels were found to be elevated in the serum of patients with renal impairment, and the protein has even been suggested as a new biochemical marker of renal insufficiency [3].

Apoptotic cell death has been observed in the course of renal injury and in clinical kidney diseases such as glomerulonephritis, acute and chronic kidney failure, diabetic nephropathy, and polycystic kidney disease [4]. Apoptosis triggered by ischemia, exogenous toxins, or endogenous factors may be the initial offense that leads to renal disease [5]. Beneficial apoptotic effects also have been observed, as in the case of tissue remodeling and recovery after renal injury. Many renal diseases are characterized by monocyte infiltration, and the clearance of these cells by apoptosis is essential to the resolution of renal inflammation. Several factors have been shown to alter the balance between survival and apoptosis in the renal microenvironment. Survival factors include insulin-like growth factor (IGF-1), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF), which have been shown to inhibit apoptosis in renal fibroblasts [6, 7].

Recent evidence suggests that arachidonic acid metabolites, namely prostaglandins, are involved in regulating cell proliferation. The prostaglandin A and J series exhibit growth inhibitory and antitumor effects [8], while prostaglandin E_2 (PGE_2), a major arachidonic acid metabolite of the mammalian kidney, has been shown to

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modulate renal hemodynamics, metabolism, water and ion transport, and sympathetic nerve activity [9, 10]. Misoprostol, a stable PGE_1 analogue, is cytoprotective against renal ischemic-induced injury in male Sprague-Dawley rats [11]. Prostaglandins also have been shown to protect the kidney from a variety of toxins, although an exact cellular mechanism remains unknown [12]. Several upstream factors of prostaglandin synthesis have been implicated in the regulation of cellular apoptosis. For example, inhibition of 85 kD phospholipase A_2 (PLA_2), the enzyme responsible for hydrolyzing membrane phospholipids into arachidonic acid, resulted in a dose-dependent inhibition of cellular proliferation [13]. Other prostaglandins such as 15-dPGJ_2, which is quickly formed from PGD_2, are known to inhibit basic fibroblast growth factor (bFGF)-induced DNA synthesis and inhibit PDGF-directed migration [14].

Since PGD_2S levels are elevated in patients with renal failure, our objective was to examine the effect of PGD_2S on LLC-PK1 cells. In addition, the effects of other prostaglandins and growth factors on PGD_2S-induced apoptosis also were determined.

METHODS

Cell culture

The pig kidney epithelial cell line LLC-PK1, a kind gift from Dr. Julia Lever (University of Texas, Houston, TX, USA), was maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% fetal calf serum, 7.5% sodium bicarbonate, 15 mmol/L HEPES, 200 mmol/L L-glutamate, 50 U/mL penicillin, and 50 μg/mL streptomycin (Life Technologies, Gaithersburg, MD, USA) at 37°C in 5% CO_2. For terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay, the cells were plated at a density of 4.0×10^4 cells/cm^2 onto Nunc Permanox eight-well chamber slides (Rochester, NY, USA). For experiments involving growth factors, the cells were serum-starved for 24 hours prior to experimental treatments in the same medium without fetal calf serum.

Detection of apoptosis

Nuclear fragmentation consistent with apoptosis was determined primarily by the TUNEL assay as previously described in Maesaka et al [15] using the ApopDetek Cell Death Assay Kit (Enzo, Farmingdale, NY, USA). Cells were scored as TUNEL positive if they appeared as condensed cells with dark nuclei. In all assays, the apoptotic index was calculated as the percentage of TUNEL-positive cells and represented at least 1000 cells counted, and was expressed as the mean ± SEM. In addition to the TUNEL assay described earlier in this article, apoptosis was confirmed by annexin V staining and electron microscopy. The annexin V apoptosis detection kit from R&D Systems (Minneapolis, MN, USA) was used according to the manufacturer's directions. Briefly, LLC-PK1 cells were plated on chamber slides as described previously and were exposed for two hours to the experimental and control samples, washed twice with cold phosphate-buffered saline (PBS), and then covered with fluorescein-conjugated annexin V and propidium iodide. The cells were incubated for 15 minutes at room temperature in the dark, and cell staining was visualized with an epifluorescence microscope (Nikon Optiphot, Melville, NY, USA) at 490 nm for annexin and 570 nm for propidium iodide. A total of at least 1000 cells were counted per condition and the apoptotic index ± SEM was calculated and converted into fold increase over basal. Electron micrograph of LLC-PK1 cells plated at 1×10^3 cells per 35-mm Falcon Petri dish (Becton Dickinson, Franklin Lakes, NJ, USA), exposed to test reagent and fixed with 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate, pH 7.2, for one hour at 4°C, was used as additional support for apoptosis versus necrosis. The cells were post-fixed in 1% buffered osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in LX112 (Ladd Research Industries, Burlington, VT, USA). En face thin sections were stained with uranyl acetate and lead citrate and examined on a Zeiss EM10 transmission electron microscope (Carl Zeiss, Inc., Thornwood, NY, USA). Apoptotic index was determined from three blocks representing different areas from each sample and examined for morphologically apoptotic cells, that is, condensed, blackened nuclei. A total of at least 200 cells were counted per condition, and the apoptotic index ± SEM was determined.

PGD_2S protein overexpression and purification

To obtain the PGD_2S for our studies, the protein was overexpressed and purified from *Escherichia coli*. The cDNA for human PGD_2S was obtained from the ATCC (#2519499), verified by sequence analysis (data not shown), and inserted in-frame into the BamHI site of vector pRSETA by standard techniques, resulting in plasmid pRSETA/PGDS. This vector is part of the XpressJ protein expression system (Invitrogen, Carlsbad, CA, USA), which is designed for high-level prokaryotic expression controlled by a T7 promoter. Expression of the inserted gene is via the induction of T7 polymerase with 2.0 mmol/L isopropyl-β-thio-D-galactopyranoside (IPTG) in BL21(DE3) *E. coli* after cells reach mid-log phase. Purification of the protein was facilitated by the polyhistidine tag at the amino terminus of the PGD_2S fusion protein and the ProBondJ resin included in the kit. Finally, the polyhistidine was removed via enterokinase and the resulting 21 kD protein was confirmed by Western analysis (data not shown), using an antibody to PGD_2S kindly provided by D.C. Cheng (Protein Chemistry Core Laboratory, Rockefeller University, New York, NY, USA).

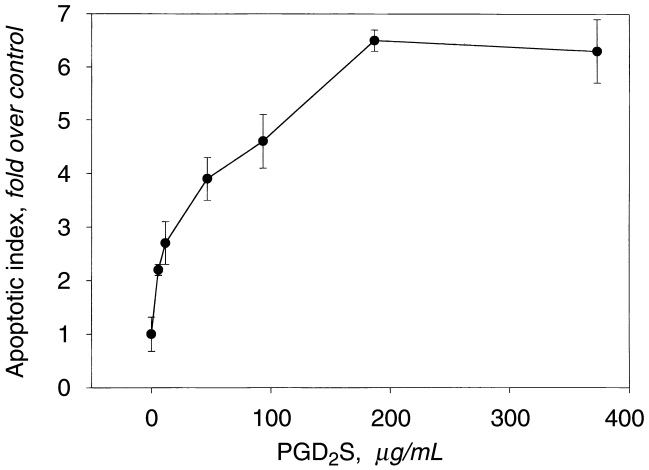

Fig. 1. Dose-response of prostaglandin D_2 synthase (PGD_2S)-induced apoptosis. LLC-PK1 cells at 75% confluence were treated with PGD_2S (0 to 400 μg/mL) for 24 hours. The TUNEL assay was used to quantitate the apoptotic index. Results are expressed as the mean ± SEM of four independent experiments each performed in duplicate and converted into fold increase over basal values.

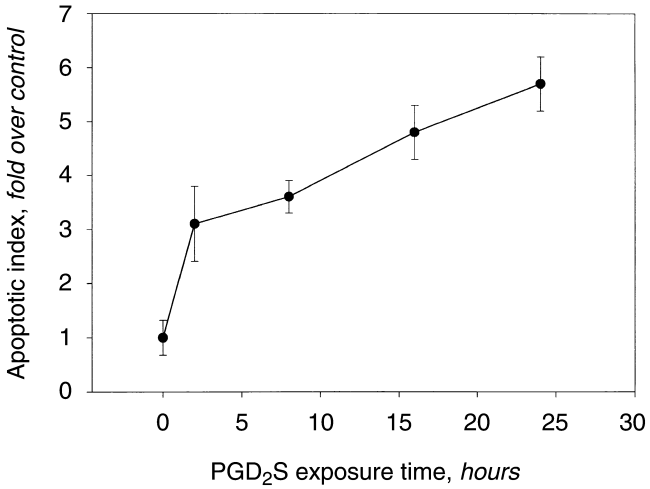

Fig. 2. Time course of PGD_2S-induced apoptosis. LLC-PK1 cells at 75% confluence were treated with PGD_2S (50 μg/mL) for 0 to 24 hours. Apoptosis was quantified using the TUNEL assay as described in the **Methods** section. Results are the mean ± SEM of four independent experiments each performed in duplicate and converted into fold increase over basal values.

Statistics

An unpaired Student *t* test was used to compare one set of experiments with another and $P < 0.05$ was deemed significant.

RESULTS

Effect of PGD_2S on LLC-PK1 apoptotic activity

The kinetics of PGD_2S incubation and apoptotic induction were measured in LLC-PK1 cells at 75% confluence by the TUNEL assay. When the cells were incubated with PGD_2S at concentrations between 0 and 400 μg/mL for 24 hours, the apoptotic index was linear up to 200 μg/mL, after which the apoptotic index reached saturation (Fig. 1). Using a fixed concentration of 50 μg/mL PGD_2S, a time course of apoptotic induction was determined. As shown in Figure 2, apoptosis was fairly linear over the 24-hour period studied. We therefore decided to use PGD_2S at a concentration of 50 μg/mL for 16 hours as our standard apoptotic induction protocol. This concentration was both convenient and close to the approximate physiological concentration observed in renal dialysis patients.

Confirmation of PGD_2S-induced apoptosis

To confirm apoptotic cell death versus necrosis, both the annexin V staining assay and electron microscopy of PGD_2S-induced cells were used. The annexin V assay exploits the fact that in apoptotic cells the phosphatidyl serine, usually localized on the interior membrane, is inverted to the outer membrane where it can bind the annexin V stain. When cells were exposed to PGD_2S (50 μg/mL) for 16 hours, the positive staining pattern of annexin V was elevated sixfold when compared with controls and matched that of the known apoptotic inducer camptothecin (Fig. 3). Propidium iodide, which stains nuclei and represents necrosis, was relatively constant for all treatments (Fig. 3). Characteristics of apoptotic cells include cell shrinkage and condensed nuclei. Figure 4 shows representative electron micrographs of LLC-PK1 cells incubated in the absence or presence of PGD_2S. As seen in Figure 4A, the control cells have round, clearly defined nuclei. However, when PGD_2S (50 μg/mL) is

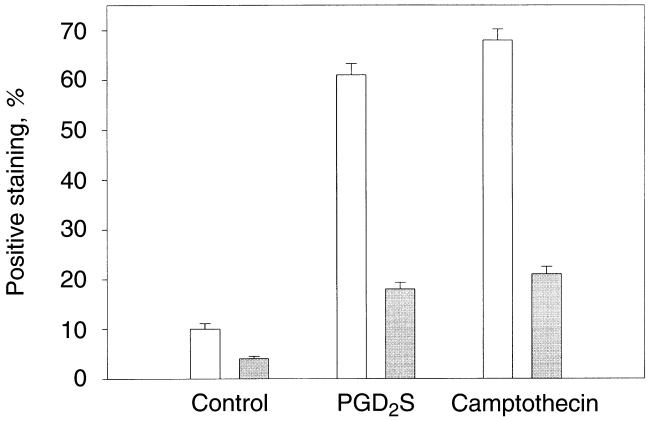

Fig. 3. Confirmation of apoptosis with annexin V staining. LLC-PK1 cells at 75% confluence were cultured as described in the **Methods** section and either 50 μg/mL PGD_2S or 10 μmol/L camptothecin was incubated with the cells for 16 hours. Cells were fixed and stained with annexin V (□) or propidium iodide (■) as described in the **Methods** section. Results are the mean ± SEM of three independent experiments each performed in duplicate.

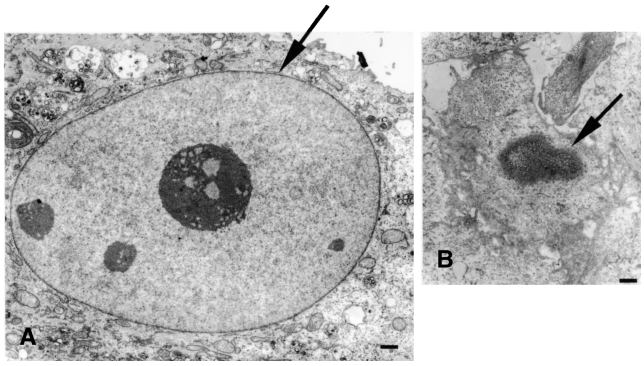

Fig. 4. Confirmation of apoptosis with electron microscopy. LLC-PK1 cells at 75% confluence were cultured as described in methods and exposed for 16 hours to either (*A*) buffer alone or (*B*) 50 μg/mL PGD_2S. The electron micrographs were prepared as described in the **Methods** section. The arrows indicate nuclear membranes and the bar in each micrograph represents 1 μm.

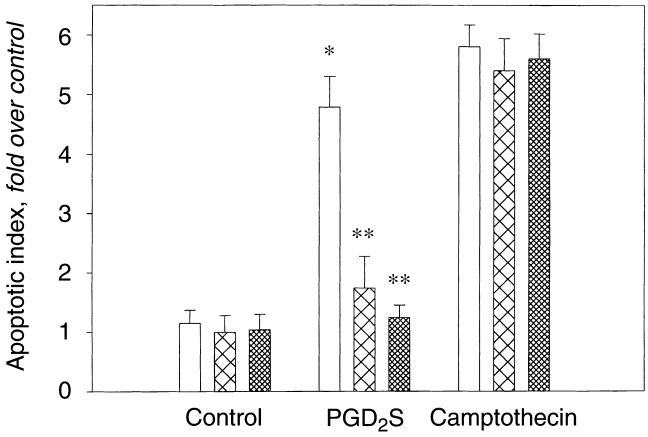

Fig. 5. Inhibition of PGD_2S-induced apoptosis with anti-PGD_2S Ab and NS398. LLC-PK1 cells at 75% confluence were cultured as described in the **Methods** section and preincubated with either 10 μmol/L NS398 or 40 μg anti-PGD_2S Ab for 15 minutes prior to incubation with either 50 μg/mL PGD_2S or 10 μmol/L camptothecin. Symbols are: (□) untreated; (▨) NS398; (▩) anti-PGD_2S antibody. The apoptotic index was determined by TUNEL assay as described in the **Methods** and the **Results** sections, and was expressed as mean ± SEM of four experiments performed in duplicate and converted into fold increase over basal values. $^*P < 0.05$ versus control; $^{**}P < 0.05$ versus untreated.

added to the cells for 16 hours, the nuclei display apoptotic characteristics such as cell shrinkage and condensed nuclei (Fig. 4B). The apoptotic index was determined by electron microscopy and was within 10% of the apoptotic index calculated using the TUNEL assay (data not shown).

Finally, PGD_2S antibody was used to inhibit the PGD_2S-induced apoptosis and to add further support to our contention that PGD_2S was indeed the agent responsible for inducing apoptosis in the LLC-PK1 cells. As shown in Figure 5, anti-PGD_2S antibody was able to inhibit the apoptosis induced by PGD_2S but had no effect on the camptothecin-induced apoptosis. The cyclooxygenase (COX)-2

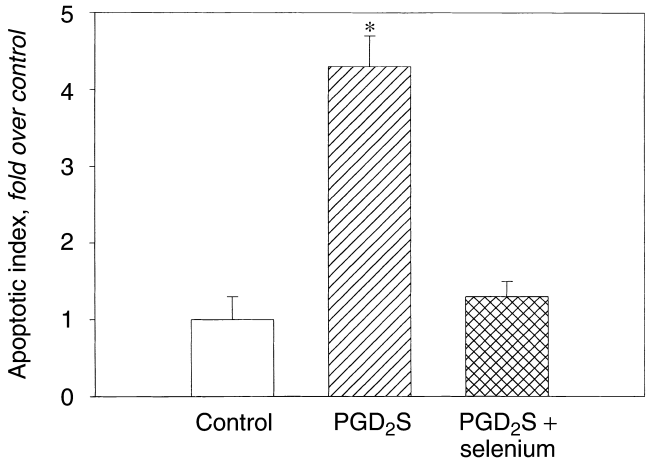

Fig. 6. Inhibition of PGD_2S-induced apoptosis with selenium. LLC-PK1 cells at 75% confluence were cultured as described in the **Methods** section and pre-incubated with 500 nmol/L sodium selenite for 15 minutes prior to incubation with 50 μg/mL PGD_2S. Apoptotic index was determined by TUNEL assay as described in the **Methods** and the **Results** sections expressed as mean ± SEM of four experiments performed in duplicate and converted into fold increase over basal. $^*P < 0.05$ versus control.

specific inhibitor, NS398, was added and returned the apoptotic values back to control levels, while having no effect on camptothecin-induced apoptosis (Fig. 5).

Inhibition of PGD_2S-induced apoptosis

Selenium compounds are known inhibitors of PGD_2S enzymatic activity when preincubated with dithiothreitol [16]. We therefore attempted to determine whether selenium would affect the apoptosis induced by PGD_2S. When LLC-PK1 cells were incubated with 50 μg/mL PGD_2S for 16 hours in the presence of 50 nmol/L selenium, apoptosis was reduced to background levels (Fig. 6). Selenium alone had no effect on the apoptotic level.

Activation of caspase activity is a characteristic feature of apoptosis. The caspase-3 inhibitor *N*-benzylcarbonyl-Asp-Glu-Val-Asp-fluoromethyl ketone (Z-DEVD) inhibited PGD_2S-induced apoptosis, whereas the control peptide, *N*-benzyloxycarbonyl-Phe-Ala-fluoromethyl ketone (Z-FA), had no effect (Fig. 7).

Since several growth factors have been shown to act as survival factors in renal epithelium [17], and certain prostaglandins have also been deemed cytoprotective [18], we sought to determine the effects of these factors on PGD_2S-induced apoptosis. When LLC-PK1 cells were serum starved for 24 hours in the presence of PGD_2S, the apoptotic activity was increased threefold over control serum-starved cells (Fig. 8). The increase in PGD_2S-induced apoptosis was inhibited with the inclusion of either IGF-1, insulin, or PDGF in the medium (Fig. 8).

The effect of other arachidonic acid-derived prostaglandins on PGD_2S-induced apoptosis also was exam-

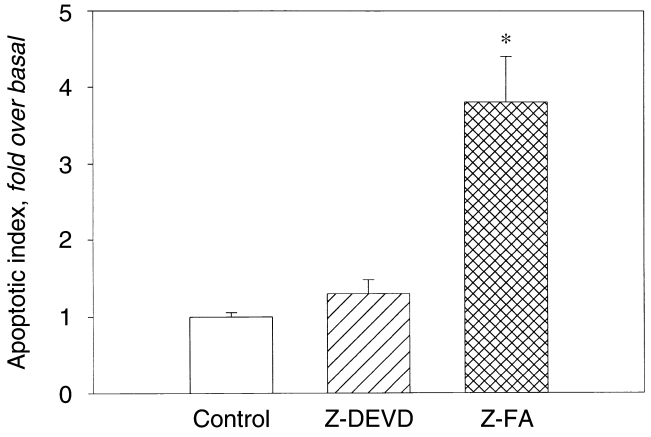

Fig. 7. Effect of caspase-3 inhibitors on PGD_2S-induced apoptosis. LLC-PK1 cells at 75% confluence were exposed to either PGD_2S (50 μg/mL) for 16 hours in the presence of either 50 μmol/L *N*-benzylcarbonyl-Asp-Glu-Asp-fluoromethyl ketone (Z-DEVD), 20 μmol/L *N*-benzyloxycarbonyl-Phe-Ala-fluoromethyl ketone (Z-FA) or dimethyl sulfoxide (DMSO) (control) and the apoptotic index measured by TUNEL assay. Apoptotic values are the means ± SEM of at least six different experiments performed in duplicate and converted into fold increase over basal. $*P < 0.05$ versus control.

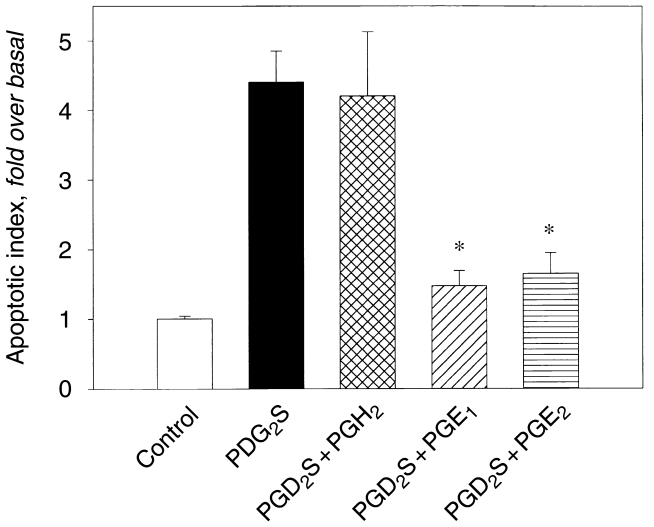

Fig. 9. Effect of other prostaglandins on PGD_2S-induced apoptosis. LLC-PK1 cells at 75% confluence were exposed for 16 hours to 50 μg/mL PGD_2S alone or in combination with 60 μg/mL PGH_2, 100 nmol/L PGE_1, 100 mol/L PGE_2, 100 nmol/L $PGF_{2\alpha}$. Apoptotic values, determined by TUNEL assay, are the means ± SEM of at least four different experiments performed in duplicate and converted into fold increase over basal values. $*P < 0.05$ versus PGD_2S alone.

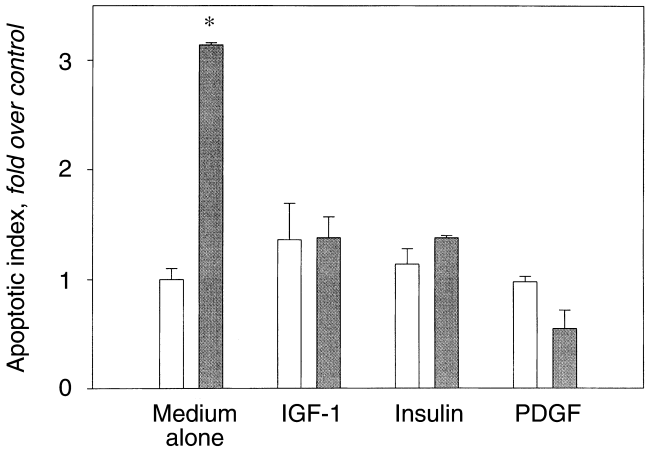

Fig. 8. Effect of growth factors on PGD_2S-induced apoptosis. LLC-PK1 cells at 75% confluence were serum-starved for 24 hours in medium without fetal bovine serum. Cells were then exposed to 20 ng/mL insulin-like growth factor-l (IGF-1), 10 nmol/L insulin, or 2.0 ng/mL platelet-derived growth factor (PDGF) for 30 minutes prior to exposure of 50 μg/mL PGD_2S and the apoptotic index calculated by TUNEL assay. Symbols are: (□) control; (■) PGD_2S. Results are the mean ± SEM of three different experiments each performed in duplicate and converted into fold increase over basal values. $*P < 0.05$ versus control.

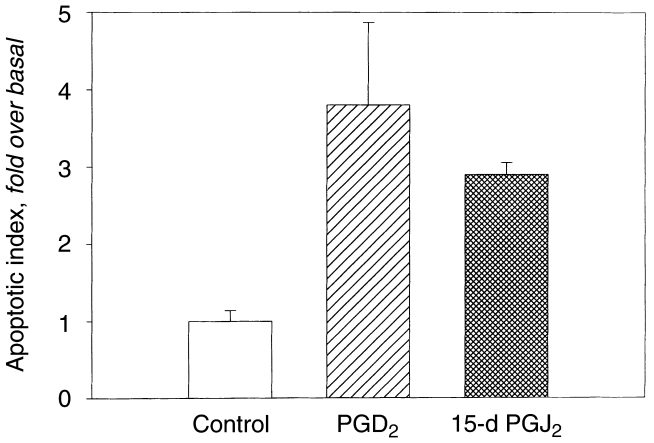

Fig. 10. Effect of PGD_2S enzymatic products on apoptosis. LLC-PK1 cells at 75% confluence were exposed for 2 hours to 15 μg/mL PGD_2 or 18 μg/mL 15-$dPGJ_2$ and the apoptotic values determined by TUNEL assay. Values are the means ± SEM of at least three different experiments performed in duplicate and converted into fold increase over basal.

ined. While the addition of PGH_2 had no effect on PGD_2S-induced apoptosis, preincubation with either PGE_1 or PGE_2 fully inhibited the apoptosis induced by PGD_2S (Fig. 9).

PGD_2S enzymatic products induce apoptosis

To elucidate further a possible mechanism of action for PGD_2S-induced apoptosis, the effects of two PGD_2S enzymatic products, PGD_2 and 15-$dPGJ_2$, were examined. As demonstrated in Figure 10, both PGD_2 and 15-$dPGJ_2$ induced a three- to fourfold increase in LLC-PK1 apoptosis after only a two-hour incubation.

PGD_2S isoforms induce apoptosis

While it was clear that our recombinant PGD_2S, isolated from *E. coli,* induced apoptosis, we decided to study the effect on apoptosis of PGD_2S isolated from different

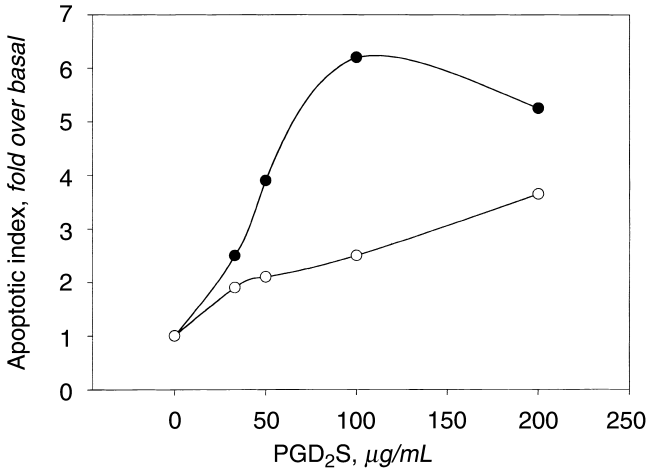

Fig. 11. Effect of PGD_2S isoforms on apoptosis. LLC-PK1 cells at 75% confluence were treated with 0 to 200 μg/mL PGD_2S isolated from human serum (●) or *Trichoplusia ni.* (○) for 24 hours. The TUNEL assay was used to quantitate the apoptotic index. Results are expressed as the mean of one experiment performed in duplicate.

sources. PGD_2S derived from human serum and recombinantly expressed in *Trichoplusia ni.* [19, 20], kindly provided by Dr. Harald Conradt, (German Research Center for Biotechnology GBF, Braunschweig, Germany), were tested for apoptotic activity by TUNEL assay. PGD_2S isolated from human serum at 100 μg/mL had a sixfold increased apoptotic induction when compared with the control, and a three- to fourfold increase in apoptotic induction over the recombinant PGD_2S, which was isolated from *T. ni.* (Fig. 11) or *E. coli* (Fig. 1), respectively.

DISCUSSION

The present study demonstrates a dose- and time-dependent apoptotic induction in LLC-PK1 cells by recombinant PGD_2S, as well as native protein isolated from human serum, using the TUNEL assay. We were confident that the cells were undergoing apoptosis for several reasons in addition to the positive TUNEL assay. The annexin V staining pattern, the physical characteristics observed by electron microscopy, and the inhibition of apoptosis observed with the caspase-3 inhibitor, Z-DEVD, all supported the notion of apoptotic cell death versus necrosis. The effect also was demonstrated in the well-characterized and immortalized human proximal tubular cell line, HK2 (data not shown).

The mechanism of PGD_2S's apoptotic action is uncertain but quite possibly involves its enzymatic activity. The fact that NS398, a COX-2–specific inhibitor (Fig. 5), and selenium, an inhibitor of PGD_2S enzymatic activity (Fig. 6), both inhibited the PGD_2S-induced apoptosis suggests that the protein is inducing apoptosis via the production of its downstream enzymatic products or by the depletion of its substrate. The fact that both PGD_2 and 15-$dPGJ_2$ induced a three- to fourfold increase in apoptosis after only two hours of exposure suggests the former (Fig. 10). Since PGD_2S is located at a vital branch point of PGH_2 distribution, the elevated serum PGD_2S levels detected in patients with kidney failure may alter the balance of PGH_2 derivatives from the cytoprotective prostaglandins such as PGE_1 and PGE_2 into PGD_2 and subsequently PGJ_2 and its derivatives. These PGJ_2 derivatives are natural ligands of peroxisome proliferator-activated receptors and have been shown to induce apoptosis in certain cell lines [2]. It is worth noting that homozygous prostaglandin endoperoxide H synthase-2 knockout mice, deficient in COX-2, have been generated and appear to develop severe nephropathy [21]. Increasing the level of PGD_2S may deplete PGH_2 levels and result in a similar consequence. Alternatively, the protein may act via a receptor or as a facilitator for the internalization of some other compound, for example, retinoids, which are known to bind to PGD_2S [1].

The influence of growth factors and certain prostaglandins on renal cell survival has been examined and is dependent on cell type [11, 12, 18, 22, 23]. PGE_1 and PGE_2 have been shown to protect renal epithelial cells from hypoxic injury [11]. Our study found PGE_1 and PGE_2 to behave in a similar cytoprotective manner, decreasing the apoptosis induced by PGD_2S, while PGH_2 had no effect (Fig. 9). Furthermore, IGF-1, EGF, and PDGF all acted as survival factors protecting LLC-PK1 cells from PGD_2S-induced apoptosis (Fig. 8). The protection from apoptosis by PDGF was not anticipated since PDGF failed to protect human and rat mesangial cells from apoptosis induced by serum-starvation and DNA damage [24]. The interplay of growth factors, as well as other prostaglandins, undoubtedly has a role in regulating PGD_2S-induced apoptosis, but it is clear that PGD_2S has a definite role in maintaining renal cell homeostasis.

The difference in apoptotic induction observed between the recombinant PGD_2S and the human protein isolated from serum suggests some sort of regulation by glycosylation since the two proteins differ by this modification. It is likely, given the distribution of PGD_2S throughout the body, that its role as an apoptotic inducer must be under some sort of regulation, which most likely is a type of glycosylation of this protein. We are currently investigating the exact mechanism by which PGD_2S induces apoptosis and the regulation by glycosylation.

It is tempting to envision other apoptotic-inducing functions for PGD_2S. Throughout embryogenesis, the PGD_2S protein expression pattern is highly specific to blood-tissue barriers such as blood-cerebrospinal, blood-testis, blood-aqueous humor, and blood-retina. PGD_2S may play a role in embryogenesis as an inducer of cellular apoptosis during tissue differentiation. The elevated PGD_2S levels found in seminal fluid may help control the relatively rapid turnover rate of sperm cells. Further-

more, PGD_2S levels are altered in the cerebrospinal fluid of patients with central nervous diseases, which again suggests a possible apoptotic role for this protein. Our unpublished data from PC12 and N-TERA-2 cells suggest a similar apoptotic function for PGD_2S in neuronal cells, and its role in some of these processes is currently under investigation.

Our current study introduces a novel role for PGD_2S as an apoptotic inducer. We speculate that the accumulation of PGD_2S in the serum of kidney failure patients, a chronic complication of kidney failure resulting from decreased kidney function, may exacerbate renal problems by the apoptosis of intrinsic renal cells, as well as the infiltrating leukocytes associated with renal disease, and contribute to the pathogenesis of renal disease. Further investigation should help clarify the exact role of PGD_2S in renal disease.

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