SUPPRESSION OF 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE mRNA CONCENTRATION, PROTEIN EXPRESSION, AND ENZYMATIC ACTIVITY DURING HUMAN URETERAL OBSTRUCTION

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Running Title: PGDH suppression in obstructed ureter

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Nonstandard abbreviations: PGDH, 15-hydroxyprostaglandin dehydrogenase; RT-PCR, reverse transcriptase-polymerase chain reaction; COX, cyclooxygenase; GAPDH, glyceraldehyde-6-phosphate dehydrogenase; PGE2, prostaglandin E2; NAD, nicotinamide adenine dinucleotide; CV, coefficient of variance; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis; TLC, thin layer chromatography; erk, extracellular regulated kinase; IL-1β, interleukin-1β; PKC, protein kinase C; TNFα, tumor necrosis factor-α; 13-HPODE, 13-hydroxyperoxyoctadecadienoic acid

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ABSTRACT

Prostanoids produce significant effects in the ureter, particularly in response to obstruction. Ureteral obstruction is associated with increased prostanoid synthesis via cyclooxygenase induction; however, prostaglandin degradation mediated by 15-hydroxyprostaglandin dehydrogenase (PGDH) has not been evaluated in the ureter. The purpose of this study was to determine if PGDH steady state mRNA, protein, and enzyme activity are altered in the human ureter during obstruction. Human ureteral segments from patients undergoing donor nephrectomy (normal segments) or ureteral stricture repair (obstructed segments) were obtained with proper informed consent. We evaluated PGDH steady-state mRNA relative to ribosomal protein S26 reference gene by RT-PCR and Vistra Green™ fluoroimaging. We determined PGDH protein content relative to glyceraldehyde phosphate dehydrogenase (GAPDH) by immunoblotting and PGDH localization by immunohistochemistry. PGDH enzymatic activity was determined by measurement of conversion of 15-hydroxy- to 15-keto-prostaglandin using thin layer chromatography separation. We found that PGDH mRNA and protein were decreased 4 to 6-fold, and enzyme activity was decreased >3-fold in obstructed human ureter relative to normal controls. PGDH was localized to the urothelial cells, with little or no expression in smooth muscle. Our results indicate that PGDH mRNA, protein and enzyme activity are suppressed in the human ureter during obstruction. Increased concentrations of prostanoids subsequent to ureteral obstruction appear to be due to decreased degradation as well as increased synthesis. Modulation of prostanoid degradation may have therapeutic relevance in obstructive disorders of the ureter.
Obstructive diseases of the ureter are among the most painful conditions experienced by humans and can have deleterious effects in the urinary tract (Gulmi et al 1999). Despite this, our understanding of ureteral physiology and function subsequent to obstruction remains limited. This gap in knowledge is a significant barrier to improved treatment of these patients. Urinary calculi are by far the most common cause of ureteral obstruction, but obstruction can also be caused by ureteral strictures, tumors, sloughed papilla, primary megaureter disease, pregnancy, vascular anomalies, and introduction of foreign bodies such as stents (Young et al 1999). Thirteen percent of Americans will experience ureteral obstruction at some point in their lifetime (Ramello et al 2000), and societal costs of this disease total over $2 billion annually (Clark et al 1995).

Prostanoid release significantly effects ureteral function through increased contractility, pressure, and pain. Prostanoids are synthesized by cyclooxygenases (COX), and our laboratory recently reported an upregulation of the inducible isoform (COX-2) during human ureteral obstruction (Nakada et al. 2002). Prostanoids mediate the pathophysiology associated with ureteral obstruction by two identified biochemical mechanisms: 1) prostanoid receptor activation resulting in increase or decrease of smooth muscle tone and contractility (Cole et al 1988); and 2) direct hypersensitization of nociceptors potentiating sensory afferent neuron stimulation by pain mediators such as substance P and bradykinin (Cesare and McNaughton, 1997). Blockade of prostanoid synthesis with nonselective COX inhibitors has been used successfully to treat severe pain associated with ureteral obstruction (Basar et al 1991), but serious side effects, including gastric ulceration, renal insufficiency, and platelet dysfunction remain significant concerns (Colletti et al 1999, Perlmutter et al 1993).

Although most investigations of prostanoids in the ureter have focused on increased prostanoid synthesis, prostanoid degradation in the ureter remains largely undescribed. The primary pathway of
prostanoid metabolism occurs in three major steps [Figure 1]: 1) dehydration of the 15-hydroxyl group; 2) saturation of the Δ13 double bond; and 3) β-oxidation (Anggard and Oliw 1981). The first step of this process (the rate-limiting step) is catalyzed by 15-hydroxyprostaglandin dehydrogenase (PGDH, Bakhle 1982). Two isoforms of PGDH have been identified and are characterized by cofactor use (Ensor and Tai 1995). Type I PGDH uses nicotinamide adenine dinucleotide (NAD+) as a cofactor and preferentially metabolizes PGE2 and PGF2α, while type II PGDH uses nicotinamide adenine dinucleotide phosphate (NADP+) as a cofactor and is relatively substrate nonspecific (Ensor and Tai 1995). The catalytic efficiency and relative expression of the NADP+-dependent isoform are considerably less than that of the NAD+ -dependent isoform. Therefore the NAD+-dependent (Type I) PGDH is considered the primary source of prostanoid metabolism in most tissues, particularly for metabolism of PGE2 and PGF2α (Chung et al 1987).

PGDH is regulated in organ systems in which precise control of prostanoid concentration is critical for homeostasis or biological function. This is particularly true in initiation of labor and regulation of menstrual cycle, inflammation, glaucoma, and encephalitis. In addition, reduction of PGDH activity in hydronephrotic kidneys is believed to play a role in modulating renal blood flow (Wong et al 1985). The purpose of this study was to determine if PGDH mRNA expression, protein expression, and activity are regulated in the human ureter in response to obstruction.
MATERIALS AND METHODS:

All chemicals were purchased from Acros/ Fisher Scientific (Pittsburgh, PA) unless otherwise stated.

Collection and preparation of human ureteral segments.

Human ureteral tissues were obtained from excess segments of ureters from patients undergoing donor nephrectomy (normal ureters) or ureteral stricture repair procedures (obstructed ureters). Tissue use was approved by the appropriate institutional review board and was obtained with informed patient consent. Ureteral segments were placed immediately in Krebs physiological salt solution [pH 7.4; composition (mM): NaCl, 119; NaH$_2$PO$_4$, 1; KCl, 4.7; CaCl$_2$, 2.5; MgCl$_2$, 0.5; NaHCO$_3$, 25; and glucose, 25] and transported to the laboratory (approximately 5 minutes). Adherent tissues were removed, and tubular ureteral segments were isolated as rings, 3-4 mm in length, weighing approximately 0.1 grams. Segments were either snap frozen in liquid nitrogen and stored at -70°C to prevent degradation of RNA or fixed in phosphate buffered formalin (10%) for immunohistochemical analysis.

Evaluation of PGDH mRNA concentrations in the ureter.

RNA extraction. Snap frozen segments were placed in 1 ml Trizol solution (acid guanidium thiocyanate phenol- GibcoBRL, Grand Island, NY) and kept at 4°C while homogenized (one minute). Homogenates were incubated at room temperature for 5 minutes, and 0.2 ml 100% chloroform was added. Homogenates were cooled to 4°C, and centrifuged at 11,000xG. The supernatant was collected, and 0.5 ml 100% isopropanol was added. After a 10-minute incubation at 20°C, samples were recentrifuged and supernatant was discarded. A 75% ethanol wash was performed and the RNA
was collected after centrifugation and evaporation of supernatant. RNA yield was quantified using a DU 640B spectrometer (Beckman-Coulter, Fullerton, CA).

**Reverse Transcription.** Extracted total RNA was added to excess Moloney murine leukemia virus reverse transcriptase (RT, Promega Corp, Madison, WI) in buffer supplied by the manufacturer along with excess random hexamers and deoxyribonucleotide triphosphates (dNTPs). After a one-minute denaturation period at 65°C, reverse transcription was performed at 37°C for 2 hours.

**Polymerase Chain Reaction.** Oligonucleotide primers for PGDH and ribosomal protein S26 were designed from the published human sequences: human PGDH, sense: ACCTCAGAAGACTCTGTTCA, antisense: CTCACACCACGTCTGGTCATAAG; human S26 (housekeeping gene), sense: CGTGCCTCCAAGATGACAAA, antisense: TAAATCGGGGTGGGGGTGTT. cDNA equivalent to 50 ng of untranscribed RNA was amplified in a 50 µl volume using 1.25U Taq DNA polymerase (Perkin Elmer, Roche Inc, NJ) in PCR reaction buffer (10 mM Tris HCl, 50 mM KCl, and 1.5 mM MgCl₂, pH=8.3) with an excess of the primers listed above and dNTPs. The following cycle parameters were employed: denaturing at 94°C for 30 seconds; annealing at 61°C for 30 seconds; and elongation at 72°C for 60 seconds. To calculate the ratio of PGDH to S26 housekeeping gene, PCR amplification of cDNA to the linear range was performed: 20, 22, 24 cycles and 25, 27, 29 cycles for S26 and PGDH, respectively. Reactions were performed using a Stratagene RoboCycler Gradient 40 (LaJolla, CA), in triplicate.
Quantification of PCR products. Following amplification, PCR products were identified by Vistra Green™ (Amersham) agarose gel electrophoresis and quantified using a Storm 860 fluoroimager and ImageQuant 5.0 software (Molecular Dynamics, Sunnyvale, CA). Band intensity was evaluated for each amplification, and ratios of intensities of PGDH / S26 were determined at the following relative number of cycles within the linear range for each cDNA: 25/20; 27/22; 29/24. Thus amplification ratios were determined at 3 points in the linear range of amplification that were consistent for obstructed and normal groups. The average ratio within the 3 points was determined and reported as one data point, with all coefficients of variation (cV) less than 0.30. The ratios for each group (obstructed and normal, n=4) were averaged and compared. Statistical evaluation was done using an unpaired student’s T-test, with p<0.05 indicative of statistical significance.

Evaluation of PGDH protein concentrations in the ureter.

Immunoblot analysis of PGDH protein concentration. Obstructed and normal ureteral segments were homogenized in protease inhibitor containing lysis buffer (150 mM NaCl, 10 mM Tris, 1 mM EDTA, 1mM phenylmethylsulfonyl flouride, and 10 µg/ml each of leupeptin, aprotinin, and antipain). Triton X-100 was added to a concentration of 1.0% (v/v) and the homogenate was incubated for 30 minutes at 4°C. The homogenate was centrifuged and the supernatant collected. Total protein concentration was determined using BCA assay (Pierce, Rockford IL). Twenty-five µg of protein extract was heat denatured in sodium dodecyl sulphate (SDS) running buffer (125 mM Tris pH 6.8, 1% w/v SDS, 10% v/v glycerol, 5% v/v β-mercaptoethanol, 0.1% w/v bromophenol blue) for 10 minutes at 100°C and resolved by SDS-polyacrylamide gel electrophoresis (PAGE, 12% polyacrylamide Tris-HCl gels, Bio-rad, Hercules, CA). Resolved proteins were transferred to nitrocellulose blotting membranes (Bio-rad) using a semidry blotter and blocked with blotto-B solution (1% w/v non-fat dry milk, 1% w/v
bovine serum albumin, 0.05% w/v NaNO₃, 0.05% v/v tween-20 in 1x phosphate buffered saline (PBS)) for at least 1 hour. Membranes were then incubated with rabbit polyclonal anti-PGDH for 18 hours (Cayman Chemical, Ann Arbor, MI; 1:2000 in blotto-B) or mouse anti-GAPDH (Biogenesis, Poole, UK; 1: 30,000 in blotto B) for 1 hour to evaluate equivalence of protein loading. The nitrocellulose blots were washed 6 times with PBS + 0.05% tween-20 and incubated with either goat anti-rabbit (PGDH blots) or goat anti-mouse (GAPDH blots) IgGs (Pierce, Rockford, IL) conjugated to horseradish peroxidase in Blotto B (minus NaNO₃) for 1 hour. Peroxidase activity was detected via chemiluminescence (SuperSignal West Femto luminol substrate and peroxide buffer-Pierce). Photoimages were analyzed by NIH image Scion software, and ratios of PGDH to GAPDH protein were determined and compared between obstructed and normal tissues. Statistical analysis was performed via unpaired student’s T-test; p-values of less than 0.05 were considered statistically different.

Immunohistochemical (IHC) analysis of protein localization. Specimens were fixed at room temperature in 10% buffered formalin and processed routinely in graded ethanol, cleared in xylene, and embedded in paraffin. The segments were imbedded longitudinally and serial cross-sections (5 microns) were obtained. Immunostaining for PGDH was performed using a 4-plus™ HRP-DAB detection kit according to the manufacturer’s instructions (Biocare Medical, Walnut Creek, CA). Briefly, slides were deparaffinized in xylene and rehydrated through immersion in ethanol gradient. Endogenous peroxidase activity was inactivated and slides were washed in tris-buffered saline (TBS) and subjected to antigen retrieval in 0.01M EDTA-80% glycerol buffer. After washing in TBS, slides were incubated with blocking solution for 30 minutes, followed by incubation with primary antibody overnight at 4°C. Primary antibody was rabbit anti-PGDH (Cayman), and the negative control was species-specific immunoglobulin G at 5 µg/ml. Slides were washed, and
antibody localization was identified using an avidin-biotin system with diaminobenzidine and peroxide as the indicator. A dark brown precipitate indicated positive antibody localization.

**Evaluation of PGDH enzymatic activity in the ureter.**

*PGDH activity assay and media extraction.* Normal and obstructed human ureteral segments (0.1 g) were incubated for 1 hour at 37°C in 1 ml Krebs buffer, with buffer changed every 15 minutes. Tissues were then incubated for 1 hour with 1 μM indomethacin (Sigma Chemical, St. Louis, MO) to inhibit endogenous prostanoid production. Buffer was replaced with fresh Krebs (1 ml) containing [3H]-PGE₂ (5,6,8,11,12,14,15-³H(N); 0.5 μCi /ml; 3 nM PGE₂; NEN biolabs, Boston, MA), 1 μM indomethacin, and tissues were incubated for 3 hours at 37°C. One vial containing buffer without tissue was used as an autooxidation control. The buffer (1 ml) was collected every hour up to 3 hours and transferred to glass stoppered tubes. HCl (1.0N, 30 μl) was added, along with 3 ml 100% ethyl acetate. The solution was thoroughly vortexed, centrifuged to achieve phase separation, and the organic phase collected and dried at 45°C under a stream of nitrogen. The product was reconstituted in 100 μl ethyl acetate and assayed via thin layer chromatography for product content. Radioactivity within the aqueous phase before and after extraction was quantified via scintillation to determine an extraction efficiency ratio. Total protein in each tissue was calculated by BCA assay in conditions recommended by the manufacturer (Pierce).

**Thin Layer Chromatography (TLC).** 20cm x 20cm aluminum backed silica gel TLC plates were pre-run in chloroform to remove impurities and air dried. Plates were spotted 1 cm from the bottom with all extracted product from the PGDH activity assay and autooxidation controls, in addition to known concentrations of [3H]-PGE₂ to derive a standard curve. Non-radioactive PGE₂
and 15-keto PGE₂ (0.10 mg/ml) were added to each plate as visualized standards. The plates were placed in TLC tanks containing the water-saturated organic phase of TLC running buffer (39% ethyl acetate, 18% isooctane, 7% glacial acetic acid, 36% water). The plate was chromatographed for 13 cm and air dried. The plates were placed in a TLC tank containing iodine crystals to reveal the PGE₂ and 15-keto PGE₂ standards. Locations on plates corresponding to identified standards were excised, placed in scintillation cocktail, and processed for quantification of radioactivity.

Quantification of PGE₂ degradation. Disintegrations per minute of each collection were compared to the standard curve of [3H]-PGE₂ and picomoles PGE₂ per ml were calculated. This value was compared to the 3 nM concentration added to the sample and a pmoles per ml reduction in [3H]-PGE₂ was calculated. The autooxidation (buffer only) reduction rate in PGE₂ was calculated and an autooxidation ratio ([3H]-PGE₂ / 3 nM at each time point) was determined. The pmoles per ml [3H]-PGE₂ reduction rate for each sample was multiplied by the autooxidation ratio, followed by the organic extraction efficiency ratio (extractions with efficiency ratios of <0.9 were not used in this study), and an adjusted pmole/ml [3H]-PGE₂ reduction rate was calculated. This was divided by the total protein in each tissue and picomoles [3H]-PGE₂ converted per gram total protein was reported. The picomoles [3H]-PGE₂/g protein converted per hour for obstructed and normal ureter was determined at 1, 2, and 3 hours of incubation, and results for obstructed and normal ureter were compared with unpaired student’s T-test.
RESULTS:

**PGDH mRNA concentrations**

In all ureteral segments analyzed, PCR amplification of S26 cDNA at 20, 22, and 24 cycles fell into a linear range of amplification. Similarly, amplification of PGDH cDNA at 25, 27, and 29 cycles produced a linear range of amplification. [Figure 2.] Amplification beyond these cycle numbers produced non-linear increases in DNA product, indicating saturation of the reaction. All PCR reactions used for quantification were completed in triplicate and repeatable with coefficient of variation values of less than 0.3. ImageQuant 5.0 analysis and ratio determination demonstrated that the ratio of PGDH mRNA concentrations to S26 in the linear ranges of amplification was 2.0 ± 0.12 in normal ureter compared to 0.67 ± 0.15 in the obstructed ureter (n=4 both groups). This difference was statistically significant (p=0.001).

**PGDH protein concentrations**

Immunoblotting of ureteral protein preparations for PGDH detected a single protein band at 29 KD, the molecular weight of PGDH. [Figure 3] Blotting for GAPDH detected a single band at 38 KD, the molecular weight of GAPDH. Obstructed ureter exhibited significantly reduced PGDH relative protein concentrations, as the ratio of PGDH/GAPDH was 1.78 (±0.22) in the normal ureter and 0.33 (±0.14) in the obstructed ureter (n=5, p=0.001).

Immunohistochemical staining of normal and obstructed ureters revealed strong positive localization of PGDH in the urothelial cells of normal ureter, while less intense detection was...
observed in the obstructed ureter. [Figure 4] PGDH was not detected in the smooth muscle or adventitia of the ureter.

**PGDH enzymatic activity**

Scintillation counting of selected regions of the gels revealed that normal ureters converted 17.7 (±4.8) pmoles of [3H]-PGE2 per gram of protein per hour in the first hour of reaction, 14.5 (±4.5) in hour 2, and 7.8 (±2.5) in hour 3. [Figure 5] This activity was repressed in the obstructed ureter to 4.5 (±2.5) pmoles/g/hr in hour 1, 2.9 (±1.1) in hour 2, and 1.5 (±0.5) in hour 3. These data were obtained in tissues pretreated with indomethacin (1µM) to prevent saturation of the cellular PGDH enzymes with endogenous prostanoids. Tissues not treated with indomethacin converted less than 10% of this amount and meaningful data were not calculated. Immunoblotting of indomethacin-treated normal and obstructed ureter showed that indomethacin had no effect on detectable PGDH protein concentrations, as the ratio of PGDH/GAPDH was 1.10 (±0.18) in normal ureters treated with indomethacin and 0.95 (±0.05) in non-treated tissues (n=3).
DISCUSSION:

Although PGDH expression and activity have been evaluated in the urinary tract previously (Chang et al 1991, Wong et al 1985), our report is the first to describe PGDH expression and activity in the ureter in response to obstruction. Our data indicate that PGDH mRNA expression, protein expression, and enzymatic activity are suppressed 3-6 fold during human ureteral obstruction. Suppression of PGDH expression occurred in all 4 ureters evaluated in mRNA analysis, all 5 ureters in immunoblotting analysis, and all 4 ureters used in enzyme activity analysis, indicating that this regulation is a consistent response of the human ureter to obstruction. The consistent suppression of PGDH mRNA, protein concentrations, and enzymatic activity suggest that PGDH repression occurs primarily at the transcriptional or posttranscriptional level, and that neither protein synthesis or enzymatic efficiency are affected.

In analysis of PGDH enzymatic activity, we used 1 μM indomethacin to inhibit prostanoid production and reduce saturation of PGDH with endogenous prostanoids, thereby potentiating the effect of PGDH on radiolabeled prostanoids. It has been reported that indomethacin can induce PGDH expression in a concentration and time-dependent manner (Frenkian et al 2001). However, we evaluated PGDH protein via immunoblot with and without 1 μM indomethacin for the three hour period of the incubation and observed no effect of indomethacin on PGDH expression. Therefore, the observed enhancement of PGE₂ metabolism in the presence of indomethacin is likely due to blockade of endogenous prostanoid production rather than induction of the enzyme, at least in the concentrations and time points used in our study.
Three additional aspects of prostanoid metabolism during ureteral obstruction remain unresolved by this study. The first is the relative oxidation/reduction (redox) state of the urothelial cells during ureteral obstruction. The effect of ureteral obstruction on the redox state of urothelial cells is undetermined, yet redox changes in intact cells can have profound effects on dehydrogenase activity in intact cells and tissues. Future work stemming from this study should include determining the redox state of urothelial cells in response to obstruction, and determining what effect redox potential has on PGDH activity in this condition. Secondly, the present study does not resolve the role of 15-ketoprostaglandin-13-reductase (13-PGR) during ureteral obstruction. While PGDH is considered the rate-limiting step in prostanoid metabolism and oxidation of the 15-hydroxy to 15-keto renders prostaglandins inactive, obstruction could regulate the expression of 13-PGR, which may have effects on overall prostanoid metabolism in urothelial cells. Finally, the transport of prostanoids into cells where PGDH resides could be regulated by ureteral obstruction. This could have effects on the overall metabolism of prostanoids, as has been observed in lipopolysaccharide-induced fever (Ivanov et al 2003).

Suppression of PGDH expression represents the second known distinct mechanism of prostanoid elevation during ureteral obstruction. We have reported previously that ureteral obstruction is associated with a 4-fold increase in cyclooxygenase (COX)-2 expression (Nakada et al 2002). The finding that prostanoid metabolism is concurrently suppressed suggests that the concentrations of biologically active prostanoids in the diseased ureter is dramatically enhanced in a bimodal fashion. Prostanoid synthesis has been a long-standing target of pharmacological intervention, and COX inhibitors have clinical efficacy for treatment of symptomatic ureteral obstruction. Our data suggest that enhancing the metabolism of active prostanoids may also be a logical target of drug action.
This report adds to the known physiological processes in which PGDH expression is regulated. The best-studied physiological processes involving PGDH regulation are pregnancy and birth. Uterine PGDH expression increases significantly throughout the course of pregnancy, most likely to inhibit excess prostanoids from inducing premature labor (Schoof et al 2001). At term, PGDH expression in uterine tissue is decreased dramatically (Patel et al 2002). The result of this repression is substantially increased prostanoid concentrations and induction of uterine contraction. Reduced PGDH is particularly apparent in the uterine ambion layer and cytotrophoblasts where PGDH appears nonexistent during delivery (Sangha et al 1994). In animal studies, PGDH expression is tightly regulated in the ovary and uterus throughout the estrous cycle (Silva et al 2000). PGDH expression appears even further suppressed in the infected uterus (Van Meir et al 1997). Infection is also associated with inhibition of PGDH in the lung (Ivanov et al 2003) and skin (Hahn et al 1999). In addition, reduced PGDH has been associated with urinary bladder carcinoma (Gee et al, 2001), further implicating prostanoid regulation as a primary factor in cancer cell proliferation. Our study is the first to identify an association between distension of a visceral tissue and decreased PGDH; this may also have implications in cancer biology because stretch and distension are known to induce proliferation of bladder cancer cells in vivo in a prostanoid-dependent manner (Park et al 1999).

Elucidation of cell signaling mechanisms of PGDH regulation in ureteral obstruction may lead to identification of potential therapeutic targets directed at maintaining PGDH levels. While the mechanisms of PGDH regulation are poorly studied, analysis of the promoter region of mouse PGDH has revealed several intriguing possibilities (Matsuo et al 1997). The promoter contains response elements for SP1, cAMP, glucocorticoids, activator proteins (AP) 1 and 2, nuclear factor interleukin (NF-IL)-6, and steroid hormones. While the role of steroid hormones in PGDH regulation has
extensively studied (Greenland et al 2000, Chang et al 1985, Tong et al 2000a) this is an unlikely mechanism of regulation in response ureteral obstruction. Because ureteral obstruction is associated with stretch and distension, as well as inflammatory and recovery events, signaling pathways involved in these processes are more plausible. Inflammatory cytokines such as interleukin (IL) -1β and tumor necrosis factor (TNF) -α repress PGDH expression; this is reversed by the anti-inflammatory cytokine IL-10 (Pomini et al 1999). The Sp1 or NF-IL6 elements of the PGDH promoter may be involved in these cascades. This possibility is strengthened by the discovery that LPS-induced fever down-regulates PGDH and prostaglandin transporters, both of which contain an Sp1 regulation element (Ivanov et al 2003). In addition, stretch and distension might elevate intracellular cAMP concentrations. Induction of cyclic nucleotides represses PGDH expression in trophoblasts (Lennon et al 1999).

Several compounds enhance PGDH expression or block PGDH activity. Cell culture models have demonstrated that phorbol ester and 1,25 dihydroxyvitamin D3 increase PGDH concentrations (Tong et al 2000b, Pichaud et al 1997). Numerous compounds have been shown to inhibit PGDH, including arachidonate-derived 13-hydroperoxyoctadecadienoic acid (13-HPODE, Sakuma et al 1993), the sulfasalazine derivative Ph CL28 (Berry et al 1985), and natural product derivatives such as thiazolidinediones, genestein, and sofalcone (Cho et al 2002, Rao et al 1997, Kobayashi et al 1992).

In summary, PGDH mRNA expression, protein expression, and enzymatic activity are suppressed 4-6 fold during human ureteral obstruction. Suppression of PGDH combined with induction of COX-2 represent two distinct mechanisms by which prostanoid concentrations are increased during ureteral obstruction. The physiological effects of increased prostanoid
concentrations include increased nociception, ureteral hypercontractility, inflammation and cell proliferation. Future research should be aimed at identification of the cellular mechanisms involved in PGDH regulation and development of specific pharmacological agents targeting PGDH.
REFERENCES


FOOTNOTES:

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FIGURE LEGENDS:

Figure 1. Mechanism of PGE2 Metabolism.
Prostanoids are metabolized readily in most tissues to inactive metabolites. In the primary cascade of prostanoid metabolism, the first step is the conversion of physiologically active 15-hydroxyprostanoids to inactive 15-keto prostanoids. This is considered the rate-limiting step in prostanoid degradation, and it is catalyzed by 15-hydroxyprostaglandin dehydrogenase (PGDH). In a secondary cascade, PGE2 can be converted to PGF2α by prostaglandin 9-keto reductase.

Figure 2. Agarose gel of S26 and PGDH RT-PCR products.
RT-PCR was performed on cDNA from normal and obstructed human ureters. PCR amplification was performed to the linear range for each gene: 20-24 cycles for S26 and 25-29 cycles for PGDH, as indicated. The ratio of PGDH at 25, 27, and 29 cycles to S26 at 20, 22, and 24 cycles was determined. Obstructed ureters exhibited a 3-fold suppression in steady-state PGDH mRNA compared to normal ureters, as the ratio of PGDH to S26 was 1.95 in normal ureter and 0.67 in obstructed ureters (n=4, p=0.001).

Figure 3. Immunoblotting of GAPDH and PGDH protein in normal and obstructed human ureter.
Obstructed and normal human ureters were homogenized and total protein was extracted and resolved via SDS-PAGE. Proteins were transferred to nitrocellulose membranes, immunoblotted using specific monoclonal anti-human GAPDH or polyclonal anti-human PGDH antibodies and species specific secondary antibodies, and visualized by chemiluminescence. GAPDH was observed at 38 KD and PGDH was observed at 29KD. Quantification of band density with scion image software indicates
that the ratios of PGDH to GAPDH was 1.78 in normal ureter and 0.33 in obstructed ureter (n=5, p=0.001).

**Figure 4. Immunohistochemistry staining of PGDH in normal and obstructed ureter.**

Normal and obstructed human ureters were fixed in formalin, serially sectioned, and mounted. Immunohistochemistry was performed using polyclonal anti-human PGDH, and DAB chromogen staining as described. Dark staining was evident in the urothelial cells of the normal human ureter. This positive localization of PGDH was considerably reduced in the obstructed human ureter.

**Figure 5. PGDH enzyme activity assay.**

PGDH enzyme activity assay was performed as described and picomoles of [3H]-PGE$_2$ converted per gram of protein per hour were calculated for each tissue as described in the text. Normal ureters converted 17.7 pmole/g/hr in the first hour, 14.5 pmole/g/hr, in hour 2, and 7.8 pmole/g/hr in hour 3. Obstructed ureters converted 4.5 pmole/g/hr in hour 1, 2.9 pmole/g/hr in hour 2, and 1.5 pmole/g/hr in hour 3. Error bars indicate SEM; p-values were calculated via unpaired students T-test-obstructed vs. normal.
Figure 1.

[Diagram showing metabolic pathways involving PGE₂ and related compounds, including reactions catalyzed by PGDH, Δ13 PGR, and 9K PGR.]
Figure 2.

Normal Ureter

Obstructed Ureter

20 22 24 25 27 29 20 22 24 25 27 29
S26 PGDH S26 PGDH
Figure 3.

**Normal Ureter**

- GAPDH 38 KD
- PGDH 29 KD

**Obstructed Ureter**

- GAPDH 38 KD
- PGDH 29 KD
Figure 4.
Figure 5.

Picomoles [3H] PGE$_2$ converted/gram protein

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p-values:
- $p=0.046$
- $p=0.028$
- $p=0.037$