p38 MAP kinase mediates mechanically induced COX-2 and PG EP4 receptor expression in podocytes: implications for the actin cytoskeleton

Louis C. Martineau, Lyne I. McVeigh, Bernard J. Jasmin, and Chris R. J. Kennedy. p38 MAP kinase mediates mechanically induced COX-2 and PG EP4 receptor expression in podocytes: implications for the actin cytoskeleton. Am J Physiol Renal Physiol 286: F693–F701, 2004. First published December 9, 2003; 10.1152/ajprenal.00331.2003.—A dynamic cytoskeleton allows podocytes to withstand significant mechanical stress on elevation of intraglomerular capillary pressure (Pgc). However, vasoactive hormones, such as prostaglandin E2 (PGE2), may challenge the integrity of the actin cytoskeleton, alter podocyte morphology, and compromise glomerular permeability. PGE2 synthesis correlates with the onset of proteinuria and increased Pgc following reduced nephron mass. We investigated the interplay among mechanical stress, cyclooxygenase (COX), E-prostanoid (EP) receptor expression, and the actin cytoskeleton, using an in vitro model of cell stretch. Immortalized mouse podocytes grown on flexible silicone membranes were cyclically stretched (5% elongation, 0.5 Hz) for 2 h. EP4 and COX-2 mRNA increased three- and sevenfold above nonstretched controls, whereas EP1 and COX-1 levels were unchanged. Six hours of stretch resulted in a threefold increase in PGE2-stimulated cAMP accumulation, a measure of EP4 receptor function, and an increase in COX-2 protein. The stretch-induced effects on COX-2/EP4 expression and EP4-induced cAMP production were attributable to p38 MAP kinase, as blockade of this pathway, but not of ERK or JNK, abrogated the response. These stretch-induced changes in expression were transcriptionally dependent as they were actinomycin D sensitive. Finally, we investigated the influence of enhanced EP4 signaling on the actin cytoskeleton. Addition of PGE2 resulted in actin filament depolymerization observable only in stretched cells. Our results indicate that key components of the eicosanoid pathway are upregulated by mechanically stimulated p38 MAP kinase in podocytes. Enhanced EP4 receptor signaling may undermine podocyte cytoskeletal dynamics and thereby compromise filtration barrier function under conditions of increased Pgc.

PODOCYTES ARE HIGHLY differentiated epithelial cells that form the final barrier to protein in the glomerular capillary bed. Normally, the podocyte foot processes that extend from each cell body counteract the pulsatile distensive forces exerted on the capillary walls within each glomerular tuft. However, pathophysiologic conditions that increase intraglomerular capillary pressure (Pgc), such as hypertension or reduced nephron mass, expose these cells to cycles of abnormal mechanical load. The podocytes are thought to respond to such forces by undergoing aberrant morphological changes, hypertension, or detachment from the glomerular basement membrane. This damage compromises the filtration barrier, thereby giving rise to proteinuria, a key predictor of progressive renal failure (3, 6, 18, 19). The pressure-induced intracellular responses underlying these deleterious events are poorly understood and are only now being characterized.

Experimental models of elevated Pgc in vivo revealed increased expression of the inducible isoform of cyclooxygenase (COX-2) in podocytes. For example, in rats with subtotal renal ablation, glomerular COX-2 expression was upregulated in mesangial cells and podocytes concomitant with increased Pgc (37). Such increased COX-2 expression might therefore translate into elevated intraglomerular prostaglandin (PG) levels capable of acting in an auto- and/or paracrine manner. PGS may compromise the effectiveness of the perselectivity barrier by inducing remodeling of the actin cytoskeleton within the podocyte foot processes through a mechanism similar to that conferred by PG-stimulated vasodilation (28).

We recently demonstrated COX-2 expression and PGE2 production in a conditionally immortalized mouse podocyte cell line (23). PGE2 is the major renal COX metabolite and can interact with at least four G protein-coupled E-prostanoid (EP) receptor subtypes designated EP1, EP2, EP3, and EP4. Bek and co-workers (4) demonstrated the expression and signaling characteristics for both EP1 and EP4 receptors in this podocyte cell line. Whether podocyte expression of COX isoforms and EP receptor subtypes is subject to mechanical stress has not been investigated.

The increase in distention forces acting on podocytes as a result of increased Pgc can be mimicked in vitro by subjecting cells grown on a flexible substratum to cyclic strain. Recent studies employing this type of model showed that podocytes respond to mechanical stimulation by reducing proliferation rates, reorganizing their actin cytoskeleton, and enhancing gene expression (10, 11, 30). In vitro mechanical stimulation therefore represents a useful experimental approach for investigating the interplay between mechanical stress, the expression of COX-2 and other components of this eicosanoid pathway, and their effects on the actin cytoskeleton.

The purpose of the present study was to characterize mechanically regulated changes in expression of COX isoforms and EP receptor subtypes, as well as mechanically dependent changes in cytoskeletal dynamics driven by PGE2 signaling. We show stretch-induced upregulation of COX-2 and EP4 receptor expression, dependent on p38 MAPK signaling. Also,
PGE2 signaling via the EP4 receptor may perturb podocyte data support that, under conditions of increased Pgc, increased the actin cytoskeleton susceptible to disruption by PGE2. Our maintaining cultures at 37 °C in media without temperature-sensitive large T-antigen. Differentiation was induced by mouse recombinant °C in RPMI culture media supplemented with 10 U/ml gated at 33 °C supplemented with 10% FBS, 100 U/ml peni-

were grown on type I collagen-coated plastic tissue culture dishes in was carried out as described in detail previously (25). Brie

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we demonstrate that increased EP4 receptor expression renders the actin cytoskeleton susceptible to disruption by PGE2. Our data support that, under conditions of increased Pgc, increased PGE2 signaling via the EP4 receptor may perturb podocyte cytoskeletal dynamics thereby undermining the integrity of the filtration barrier.

MATERIALS AND METHODS

Cell culture and mechanical stimulation. Culture of conditionally immortalized mouse podocytes, kindly provided by Dr. P. Mundel, was carried out as described in detail previously (25). Briefly, cells were grown on type I collagen-coated plastic tissue culture dishes in RPMI-1640 medium supplemented with 10% FBS, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Podocytes were routinely propagated at 33°C in RPMI culture media supplemented with 10 U/ml mouse recombinant γ-interferon to promote the expression of the temperature-sensitive large T-antigen. Differentiation was induced by maintaining cultures at 37°C in media without γ-interferon for 7–14 days. Cell culture materials were purchased from Life Technologies (Burlington, ON). Mechanical stimulation of cells was achieved using vacuum-based Flexercell FX-4000T and collagen I-coated BioFlex culture plates (Flexcell International, Hillsborough, NC). For cell-stretching experiments, protocols and conditions previously employed by others (10, 30) were adapted. Briefly, differentiated cells were trypsinized and transferred to the flexible silicone membranes of 5% at a frequency of 0.5 Hz for 10 min to 24 h. Control cells (designated as nonstretched) were cultured under identical conditions but were not exposed to stretch. Actinomycin D (4 μg/ml; Sigma) and MAPK inhibitors SB-202190, SP-600125, and PD-98059 (Tocris, Ellisville, MO) were used at 25 μM in RPMI-1640 + 0.1% FBS, with a 30-min incubation before mechanical stimulation experiments.

Real-time RT-PCR. RNA was extracted from differentiated podocytes seeded on BioFlex plates, serum-starved overnight, and cyclically stretched to 5% elongation at 0.5 Hz for 10 min, 6 h, or 24 h, as well as time-matched, nonstretched controls, were harvested by rinsing twice and scraping in ice-cold PBS. Cells were pelleted at 12,000 g, resuspended, and sonicated in RIPA lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 0.5 mM PMSF) with a protease inhibitor cocktail (pep-

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>COX-1</td>
<td>5′-CCA GAA CCA GGG TGT CAG TCT-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-GTA GCC CTT GCG AGT ACA ACT-3′</td>
</tr>
<tr>
<td>Probe</td>
<td>6FAM-GCC TTT GGC CTC GAC AAC TAC GAG TG-TAM</td>
</tr>
<tr>
<td>EP4</td>
<td>5′-GGT TGT GCC TCT ACT TCT TTC A-3′</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′-TGG GAG GCA CTT GCA TTG A-3′</td>
</tr>
<tr>
<td>Probe</td>
<td>6FAM-GGT GCA AGA TCC ACA GCC TAC CAA AAC A-TAM</td>
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Table 1. Sequences of PCR primers and TaqMan probes used in this study

Mix Reagents (Applied Biosystems, Branchburg, NJ) and an ABI Prism 7000 Sequence Detection System according to a previously reported protocol (23, 24). Reactions were carried out using 50 ng of total podocyte RNA under the following conditions: 48°C for 30 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Primers and TaqMan probes for each target are listed in Table 1. Specificity of primers and probe sequences was verified against the National Center for Biotechnology Information database. Reactions yielded single-amplicon products of predicted size. Values were normalized to GAPDH mRNA levels in each sample as determined by a TaqMan Rodent GAPDH Control Reagent kit (Applied Biosys-

Western immunoblotting. Differentiated podocytes seeded on BioFlex plates, serum-starved overnight, and cyclically stretched to 5% elongation at 0.5 Hz for 10 min, 6 h, or 24 h, as well as time-matched, nonstretched controls, were harvested by rinsing twice and scraping in ice-cold PBS. Cells were pelleted at 12,000 g, resuspended, and sonicated in RIPA lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 0.5 mM PMSF) with a protease inhibitor cocktail (pep-

Fig. 1. Stretch increases cyclooxygenase-2 (COX-2) and E-prostanoid (EP4) receptor mRNA expression. Differentiated podocytes cultured on flexible silicone membranes were serum-starved overnight and then mechanically stimulated for 2 h with +5% equibiaxial surface elongation applied cyclically at 0.5 Hz with a sinusoidal waveform. Total RNA was extracted and analyzed by real-time RT-PCR using specific TaqMan primer/probe sets as indicated. Values have been normalized to GAPDH content and are presented as fold of respective nonstretched controls. *P < 0.01 vs. respective nonstretched control (n = 7).
RNA was extracted and analyzed by real-time RT-PCR using specific primer/probe sets for either COX-2 (A) or Ep4 receptor (B). Values have been normalized to GAPDH content and are presented as fold of respective non-stretched controls. *P < 0.05 vs. nonstretched control (n = 3).

**RESULTS**

Stretch increases COX-2 and EP4 receptor mRNA expression. To mimic the pressure cycles experienced by podocytes in vivo, a conditionally immortalized mouse podocyte cell line was cultured on flexible collagen I-coated silicone membranes and subjected to 5% biaxial mechanical strain at 0.5 Hz for 2 h, and the mRNA expression of EP receptor subtypes, COX-1, and COX-2 was determined by real-time RT-PCR. The degree of stretch was chosen to approximate pressures experienced by podocytes in vivo (29, 32). Stretch increased both COX-2 (3.3 ± 0.6-fold of nonstretched control, P < 0.01) and EP4
before approaching baseline levels at 24 h of continuous response to 8% stretch (data not shown).

stretch for 2 h, and maximal induction was observed in response to stretch. Alternatively, these modest effects in addition to stretch might be required to realize the potential of COX-2 and EP4 receptor mRNA expression in both COX-2 and EP4 receptor mRNA expression (Fig. 3).

**Stretch increases COX-2 protein expression.** To determine whether increased COX-2 mRNA levels were followed by elevated protein expression, podocytes were stretched for 6 h and the cell lysates were assayed by Western blot analysis for immunoreactive COX. Stretch increased the expression of COX-2 protein following 6 h of stretch, whereas COX-1 protein expression was unchanged by stretch (Fig. 4). Interestingly, mechanical stretch elicited a minor increase in both arachidonic acid and PGE2 levels (i.e., <25% above non-stretched controls, data not shown), implying that other factors in addition to stretch might be required to realize the potential of COX-2 expression. Alternatively, these modest effects might be attributable to the culture conditions employed.

**Stretch increases PGE2-mediated cAMP synthesis.** To determine whether increased EP4 receptor mRNA levels were followed by elevations in functional receptor expression, regulation at the transcriptional or posttranscriptional level. To determine whether inhibition of transcription would block the stretch-induced expression of COX-2 and/or EP4 receptor mRNA, podocytes were incubated with actinomycin D (4 μg/ml) and stretched for 2 h. Treatment with actinomycin D completely abolished the stretch-induced increase in both COX-2 and EP4 receptor mRNA expression (Fig. 3).

**Fig. 5.** Stretch increases PGE2-mediated cAMP synthesis. Differentiated podocytes cultured on flexible membranes were serum-starved overnight and then cyclically stretched 5% for 6 h. Cells were then preincubated for 30 min with 20 μM indomethacin + 0.5 mM isobutylmethylxanthine (IBMX) and subsequently incubated with vehicle or 1 μM PGE2 for 10 min. The intracellular cAMP content was determined from cell extracts by RIA and the total cellular protein was determined by the Bradford method. Values are presented as pmol cAMP/mg cellular protein. *P < 0.01 vs. nonstretched control. **P < 0.001 vs. nonstretched + PGE2 (n = 4).

**Fig. 6.** Stretch-induced COX-2 and EP4 expression is blocked by the p38 MAP kinase inhibitor SB-202190. Differentiated podocytes cultured on flexible membranes were serum-starved overnight and then cyclically stretched 5% for 10 min, 2 h, or 6 h in the presence or absence of p38, MEK, or JNK MAP kinase inhibitors (SB-202190, PD-98059, and SP-600125, respectively; 25 μM each). A: total RNA extracted from cells stretched for 2 h (Str) and from time-matched, nonstretched controls (NS) was analyzed by real-time RT-PCR using specific TaqMan primer/probe sets as indicated. Values have been normalized to GAPDH content and are presented as a proportion of the stretched value. *P < 0.01 vs. nonstretched control (n = 3). B: immunoblotting with an anti-COX-2 antibody was performed on lysates of cells stretched or nonstretched for 6 h in presence or absence of inhibitors. Shown is a representative blot from 1 of 3 separate experiments. Densitometry was performed to quantify changes in expression and was normalized to actin. *P < 0.05 vs. nonstretched control. C: determination of PGE2-stimulated cAMP production was performed in cells stretched or nonstretched for 6 h in presence or absence of inhibitors. After this period, cells were incubated for 30 min with 20 μM indomethacin + 0.5 mM IBMX and subsequently incubated with vehicle or 1 μM PGE2 for 10 min. Intracellular cAMP content was determined from cell extracts by RIA and the total cellular protein was determined by modified Bradford method. Values are presented as pmol cAMP/mg cellular protein. *P < 0.001 vs. vehicle nonstretched + PGE2. **P < 0.001 vs. vehicle stretched + PGE2 (n = 5). D: verification of stress-induced phosphorylation of p38 MAP kinase and subsequent inhibition by SB-202190. Cells were preincubated in the presence or absence of the MAP kinase inhibitors for 30 min and subsequently stretched at 5% for 10 min. Lysates (50 μg protein, n = 2) were analyzed by Western blot analysis using a rabbit anti-phospho-p38 antibody (1:500).

**Fig. 4.** Stretch increases COX-2 protein content. Differentiated podocytes cultured on flexible membranes were serum-starved overnight and then cyclically stretched 5% (Str) or were not stretched (NS) for 6 h. Western immunoblotting of cell lysates (50 μg protein) was performed using an antibody specific for COX-2 (middle). Blots were then sequentially stripped and re-probed first with an anti-COX-1 antibody (top) and then with an anti-actin antibody (bottom). Blots shown are representative of 3 independent experiments.

**Fig. 3.** Time course of stretch-induced COX-2 and EP4 receptor upregulation. Stretch-induced upregulation of COX-2 and EP4 receptor mRNA levels was found to peak at 2 h of continuous cyclical 5% stretch (P < 0.001 vs. nonstretched control), before approaching baseline levels at 24 h of continuous cyclical stretch (Fig. 2, A and B). COX-2 and EP4 mRNA levels were significantly increased with as little as 1% cyclical stretch for 2 h, and maximal induction was observed in response to 8% stretch (data not shown).

**Induction of COX-2 and EP4 expression by stretch is blocked by actinomycin D.** The observed increases in COX-2 and EP4 receptor mRNA levels may derive from changes in receptor (7.3 ± 1.8-fold of control, P < 0.01) mRNA expression (Fig. 1). In contrast, mRNA levels of COX-1 and the EP1 receptor subtype were unchanged. Neither EP2 nor EP3 receptor message was detectable, regardless of the application of stretch.

**Time course of stretch-induced COX-2 and EP4 receptor upregulation.** Stretch-induced upregulation of COX-2 and EP4 receptor mRNA levels was found to peak at 2 h of continuous cyclical 5% stretch (P < 0.001 vs. nonstretched control), before approaching baseline levels at 24 h of continuous cyclical stretch (Fig. 2, A and B). COX-2 and EP4 mRNA levels were significantly increased with as little as 1% cyclical stretch for 2 h, and maximal induction was observed in response to 8% stretch (data not shown).

Stimulatory increments in cyclic GMP (cGMP) and cAMP levels were associated with COX-2 and EP4 receptor mRNA expression (Fig. 3).
PGE_2-induced cAMP production was assayed following stretch. The EP_4 receptor subtype is coupled to adenyl cyclase through a stimulatory G protein (13). Cells cyclically stretched 5% for 6 h, followed by incubation with 1 μM PGE_2 for 10 min, yielded threefold more intracellular cAMP compared with nonstretched cells treated with PGE_2 (P < 0.001; Fig. 5).

*Induction of COX-2 and EP_4 expression by stretch is blocked by p38 MAP kinase inhibition. The p38, ERK, and JNK families of MAP kinases are activated by mechanical stress in various cell types, including mesangial cells (14, 15, 27), rat glomerular epithelial cells (unpublished data), and mouse podocytes (29). To determine whether these pathways are required for stretch-induced COX-2 and EP_4 receptor

\[ \text{COX-2 mRNA levels (arbitrary units)} \]

\[ \text{COX-2 protein (nanomoles/mg cell protein)} \]

\[ \text{COX-2 protein (nanomoles/mg cell protein)} \]

\[ \text{p38 MAP kinase inhibition} \]
expression in podocytes, COX-2 mRNA and protein, as well as EP4 receptor mRNA and PGE2-induced cAMP production, were assessed in cells cyclically stretched in the presence or absence of either p38 inhibitor (SB-202190; 25 μM), MEK inhibitor (PD-98059; 25 μM), or JNK inhibitor (SP-600125; 25 μM). The p38 inhibitor significantly reduced the stretch-induced COX-2 and EP4 receptor mRNA expression (P < 0.01 vs. stretched vehicle control; Fig. 6A) and similarly reduced stretch-induced COX-2 protein levels (P < 0.05 vs. stretched vehicle control; Fig. 6B) and PGE2-stimulated cAMP production (P < 0.01 vs. stretched vehicle control; Fig. 6C). Inhibitors of the ERK or JNK pathways had no statistically significant effects, although PD-98059 did partly reduce EP4 receptor mRNA and COX-2 protein induction following stretch. Consistent with these findings, podocytes that were stretched for 10 min exhibited increased phosphorylation of p38 MAPK, as detected by an antibody that specifically recognizes the phosphorylated/activated form of the enzyme (Fig. 6D) (31). Phosphorylation could be prevented by the p38 MAPK inhibitor (SB-202190) but not by the JNK inhibitor (SP-600125). A slight reduction in phospho-p38 levels was observed in cells treated with the MEK inhibitor (PD-98059), suggesting that this compound might exert subtle, yet nonspecific effects, on p38 MAPK activation.

PGE2 induces actin stress fiber dissociation following mechanical stress. It has been suggested that vasoactive eicosanoids such as PGE2 can alter the morphology of glomerular podocytes, thereby modifying the permeability barrier (8, 20, 21). Such effects likely involve modification of the podocyte actin cytoskeleton. To determine whether increased EP4 receptor expression and signaling can influence actin cytoskeletal dynamics, murine podocytes were cyclically stretched for 6 h and then stimulated with PGE2 (1 μM, 90 min). Actin stress fibers were visualized in fixed/permeabilized cells using FITC-conjugated phalloidin under fluorescence microscopy. The elaborate network of actin stress fibers characteristic of this cell line remained intact following mechanical stimulation, or in response to PGE2 in nonstretched cells (Fig. 7A). However, PGE2 stimulation of stretched cells resulted in a dramatic loss of actin stress fiber organization, as exhibited by a diffuse pattern of fluorescence (Fig. 7A) in >70% of stretched podocytes (Fig. 7B).

DISCUSSION

By employing an in vitro model of cell stretch, the present study investigated the interplay among mechanical stress, COX and EP receptor expression, and the actin cytoskeleton in podocytes. In vivo, the podocyte foot processes are subjected to continuous cycles of mechanical strain as these epithelial cells counteract the distensive forces acting on the glomerular wall. However, under conditions of glomerular hypertension, an increased Pgc is coupled with exposure to vasoactive hormones, such as locally produced prostaglandins, may challenge the ability of the foot processes to withstand mechanical stress, thereby resulting in proteinuria (26). Support for this notion comes from the finding that both mesangial cell and podocyte COX-2 expression are upregulated following increases in $P_{gc}$ brought about by subtotal renal ablation in rats (37) and that the associated proteinuria can be prevented by inhibiting COX-2 (36). Our present data are consistent with these observations and show that podocytes cultured in vitro respond to mechanical stress by enhancing the expression of both COX-2 and the EP4 receptor but not that of COX-1, EP1, EP2, or EP3, thereby suggesting that the EP4 receptor is a key mediator for COX-2-derived PGE2 in the glomerulus under conditions where $P_{gc}$ is elevated. On the other hand, the transient nature of this upregulation (Fig. 2) and the small increase in PGE2 synthesis in cultured cells suggest that other factors encountered in vivo may be required to sustain the expression of COX-2 and/or the EP4 receptor following changes in mechanical load.

Mechanical forces applied to podocytes can have significant impact on gene expression (11) and can reduce proliferation (30). Such effects suggest that numerous signaling cascades are activated in podocytes by mechanical stress. We investigated the signal transduction pathway by which stretch elevates EP4 and COX-2 expression. Our findings implicate p38 MAP kinase activation in mediating the levels of these gene products. The p38 MAP kinase pathway regulates COX-2 expression in a number of cell types in response to a variety of stimuli. For example, Fauré et al. (12) showed that IL-1β-stimulated COX-2 expression is p38 MAP kinase dependent in fibroblasts, whereas Cheng et al. (7) demonstrated that extra-cellular Cl– can increase COX-2 expression in a p38-dependent manner in rabbit cortical thick ascending limb cells. Mechanical stretch upregulates COX-2 expression in mesangial cells via protein kinase C, although a role for p38 MAP kinase was not ruled out in those studies (1). Our results are the first to link mechanical stress with COX-2 expression via the p38 MAP kinase signaling cascade.

Although a variety of factors have been shown to regulate COX-2 levels, little is known regarding those affecting EP4 receptor expression. Our data are the first to implicate p38 MAP kinase activation in EP4 receptor expression initiated by mechanical stress. The fact that both COX-2 and EP4 share similar time courses and require p38 activation suggests the involvement of concurrent regulatory mechanisms. Actinomycin D completely abrogated stretch-induced COX-2 and EP4 receptor mRNA increases, thereby suggesting that control of expression is exerted at a transcriptional level rather than posttranscriptionally (i.e., increased mRNA stability or translational efficiency). Future experiments will elucidate the specific gene promoter elements subject to mechanical stretch. An initial examination of the COX-2 and EP4 promoter regions (2) reveals the presence of several shared putative cis-acting elements, including NF-IL6 (C/EBPδ) (35), c-Jun through v-Src (39–41), C/EBP6 (16), and NF-κB (42).

The elaborate morphology of the podocyte is upheld by a cytoskeletal framework comprising a number of components including actin. The actin network in each podocyte is dynamic, and remodeling has been shown to occur in response to mechanical stimulation. Studies by Endlich et al. (10) show that podocytes cultured in vitro rearrange their actin cytoskeleton into radial stress fibers in response to chronic application of mechanical stress. Our results indicate that actin stress fiber organization observed in stretched cells is disrupted by PGE2. This EP4 receptor-mediated effect on the podocyte cytoskeleton might hinder adaptations to mechanical stress and is consistent with a growing body of evidence suggesting that podocyte cell morphology.

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and actin assembly are influenced by cAMP (9). Early studies of rat glomerular epithelial cells grown on plastic demonstrated that cAMP mimetics cause rearrangement of the actin cytoskeleton into a distinct stellate pattern (33). Our findings are therefore highly relevant because podocyte foot processes are known to contain high concentrations of actin, and several reports documented an abnormal distribution and disaggregation of podocyte actin microfilaments during the development of foot process effacement in vivo (17, 22, 38). Upregulation of EP₄ receptor signaling in podocytes may therefore play a critical role in pressure-induced morphological changes associated with proteinuria. However, we must temper such conclusions because the progression of podocyte foot process lesions in some diseases can yield an enrichment of F-actin density. For example, a rat model of focal and segmental glomerulosclerosis is characterized by effacement and proteinuria that correlate with increased foot process F-actin content (34). Similarly,
the role of PGE_2 in modulating podocyte structure/function is also likely to be subject to the prevailing disease etiology, as some studies showed that oxidative stress-mediated podocyte damage can be attenuated by this eicosanoid through its induction of Stra13 (5). In light of these observations, future studies should be aimed at more clearly defining the interactions between cytoskeleton and PGE_2/EP receptors in vivo, specifically in disease contexts involving PGE_2 changes, while taking into account other signaling influences.

In summary, our data demonstrate that both EP_2 receptor and COX-2 expression in mouse podocytes are upregulated by mechanical stimulation. Enhanced PGE_2 signaling may be a facilitating event for morphological changes and might directly influence podocyte function under pathophysiological conditions that promote PGE_2 synthesis.

ACKNOWLEDGMENTS

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