Characterization of Prostanoid Receptors in Podocytes

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Abstract. Prostaglandins participate in the regulation of important glomerular functions and are involved in the pathogenesis of glomerular diseases. This study investigates the influence of prostaglandins on membrane voltage, ion conductances, cAMP accumulation, and cytosolic calcium activity (\([Ca^{2+}]_i\)) in differentiated podocytes. Prostaglandin \(E_2\) (\(PGE_2\)) caused a concentration-dependent depolarization and an increase of the whole cell conductance in podocytes (\(EC_{50} \approx 50 \text{ nM}\)). Compared with \(PGE_2\), the \(EP_2/EP_3/EP_4\) receptor agonist 11-deoxy-\(PGE_1\) caused an equipotent depolarization, whereas the DP receptor agonist BW 245 C, the \(EP_1/EP_3\) receptor agonist sulprostone, and the IP receptor agonist iloprost were at least 100 to 1000 times less potent than \(PGE_2\). The \(EP_2\) receptor agonist butaprost did not change membrane voltage of podocytes. The depolarizing effect of \(PGE_2\) was increased in an extracellular solution with a reduced \(Cl^-\) concentration (from 145 to 32 \(\text{mM}\)). \(PGE_2\) and the prostanoid agonists, but not the IP receptor agonist iloprost and the \(EP_2\) receptor agonist butaprost, induced a time- and concentration-dependent cAMP accumulation in podocytes. In fura-2 fluorescence experiments, \(PGE_2\), sulprostone, \(PGF_2\alpha\), fluprostenol (a potent FP agonist), and U-46619 (a selective thromboxane \(A_2\) agonist) induced a biphasic increase of \([Ca^{2+}]_i\) in 60 to 80% of podocytes. In reverse transcription-PCR studies, podocyte mRNA for the \(EP_1\), \(EP_4\), FP, and TP receptor could be amplified. These data indicate that in podocytes, \(PGE_2\) regulates distinct cellular functions via the \(EP_1\) and \(EP_4\) receptor, thereby increasing \([Ca^{2+}]_i\) and cAMP, respectively. Furthermore, \(PGF_2\alpha\) and U-46619 increase \([Ca^{2+}]_i\) via their specific receptors.

Within the kidney, prostaglandins have been implicated to regulate important cellular functions in a variety of different cell types through specific prostanoid receptors. They influence glomerular hemodynamics by increasing GFR and renal blood flow, especially under conditions with an increased vascular tone (1,2). Their effects on GFR may partly be mediated by a change of the ultrafiltration coefficient (\(K_f\)). In this regard, it has been assumed that prostaglandins antagonize vasoactive hormone-induced mesangial cell contraction, which might lead to a decrease of \(K_f\) (3,4).

Knowledge about the influence of prostaglandins on cellular functions of the cells that form the glomerular filtration barrier, i.e., the glomerular endothelial cell and the podocyte, is limited. Podocytes play a crucial role as a filter restricting the passage of macromolecules, and they maintain a large filtration surface through the slit membranes (5). It has been speculated that they might modify \(K_f\) by contraction or dilation of their foot processes (6). Under pathologic conditions, podocytes are the target cell of injury in several forms of glomerular injury, such as minimal change and membranous nephropathy, and focal segmental glomerulosclerosis (7). Podocytes are also believed to initiate and maintain the progression of glomerulosclerosis after subtotal nephrectomy, a model of chronic renal failure (8).

An imbalance of prostanoid action seems to play a role in glomerular diseases, which are at least in part induced by podocyte injury. Changes of prostanoid action have been reported in several glomerular diseases such as membranous nephropathy (9), toxin-induced glomerular injury (10), and development of glomerulosclerosis after subtotal renal ablation (11,12).

Because prostanoid-mediated changes of podocyte function might be involved in the pathogenesis of these glomerular diseases, we attempted to investigate whether prostanoids influence cellular functions of differentiated cultured podocytes, and we characterized the prostanoid receptors involved.

Materials and Methods

Cell Culture

In the present study, we used conditionally immortalized mouse podocyte clone cells harboring a temperature-sensitive mutant of immortalizing SV-40 large T-antigen coupled with a promoter whose activity is enhanced by \(\gamma\)-interferon (13). Podocytes were maintained in RPMI 1640 (Life Technologies, Eggenstein, Germany) supplemented with 10% fetal calf serum (Boehringer Mannheim, Mannheim, Germany), 100 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin (Life Technologies) in a humid atmosphere with 5% \(CO_2\). To propagate podocytes, the culture medium was supplemented with 10 U/ml mouse recombinant \(\gamma\)-interferon (Sigma, Deisenhofen, Germany).
enhance the expression of T-antigen, and cells were cultivated at 33°C (permissives conditions). To induce differentiation, podocytes were cultured on type I collagen (Biochrom, Berlin, Germany) at 37°C without γ-interferon (nonpermissive conditions) and with 1% fetal calf serum for 2 to 3 wk. Arborized cells were immunologically characterized and stained positive for the Wilms’ tumor antigen and synaptopodin, a characteristic marker for podocyte foot processes in vivo and in vitro (13,14). Podocytes between passages 15 and 25 were used in all experiments.

**Patch-Clamp Experiments**

The patch-clamp method used in these experiments has been described in detail (15,16). In brief, podocytes were placed in a bath chamber on the stage of an inverted microscope, kept at 37°C, and were superfused with a phosphate-buffered Ringer’s-like solution. To vary free Ca$^{2+}$ activity, the solutions were prepared according to established techniques with ethyleneglycol-bis(β-aminooxythyl)-N,N,N′,N′-tetra-acetic acid and nitrilotriacetic acid as Ca$^{2+}$ buffers. The Ca$^{2+}$ activity was calculated from a standard equation and was determined with a Ca$^{2+}$-selective electrode (Radiometer, Copenhagen, Denmark). In ion replacement studies, the extracellular Na$^+$ or Cl$^-$ was replaced by N-methyl-d-glucamine (NMDE) or glucuronate respectively. The patch pipettes were filled with a solution containing (in mM): 95 K-gluconate, 30 KCl, 4.8 NaHPO$_4$, 1.2 NaH$_2$PO$_4$, 0.73 CaCl$_2$, 1.03 MgCl$_2$, 1 ethyleneglycol-bis(β-aminooxythyl)-N,N′,N′-tetra-acetic acid, and 5 d-glucose, pH 7.2; Ca$^{2+}$ activity 10$^{-7}$ M. The patch pipettes had an input resistance of 2 to 3 MΩ. A flowing (10 μl/h) KCl (2 M) electrode was used as a reference. The data were recorded using a patch-clamp amplifier (Fröbe and Busche, Physiologisches Institut, Freiburg, Germany) and continuously displayed on a pen recorder. The access conductance ($g_a$) was monitored in most of the experiments by the method recently described (16). The membrane voltage ($V_m$) of the cells was recorded continuously using the current clamp mode of the patch-clamp amplifier. To obtain the whole cell conductance ($G_w$), the voltage of the respective cell was clamped in the voltage clamp mode ($V_c$) to $V_m$. Starting from this value, the whole cell current was measured by depolarizing or hyperpolarizing $V_c$ in steps of 10 mV to ±40 mV. $G_m$ was calculated from the measured whole cell current ($I$), $G_a$ and $V_c$ using Kirchhoff’s and Ohm’s laws.

**Measurements of Intracellular cAMP**

Podocytes were cultured in 6-well plates. They were kept at 37°C and rinsed with a physiologic Ringer’s type-solution. After preincubation with 0.5 M 3-isobutyl-1-methylxanthine (Research Biochemicals Inc., Cologne, Germany) for 5 min, cells were exposed to the added agents. To terminate the assay, the supernatants were rapidly removed and cells were rinsed with ice-cold ethanol (70 g/L). After an ethanol extraction, cAMP concentrations were measured with an enzyme-linked immunosorbent assay (Amersham Buchner, Braunschweig, Germany).

**Measurements of Intracellular Ca$^{2+}$ Activity**

Measurements of intracellular Ca$^{2+}$ activity ([Ca$^{2+}$]) with the Ca$^{2+}$ sensitive dye fura-2 (Sigma) were performed in podocytes on an inverted fluorescence microscope (17). The system allows fluorescence measurements at the single cell level at three excitation wavelengths. The field of measurement could be chosen by an adjustable pinhole between 2 and 300 μm diameter. A time resolution of up to 200 Hz was achieved using a high-speed filter wheel and a single photon counting tube (Hamamatsu H63460-04, Herrsching, Germany). The auto fluorescence signal of cells, which had not been loaded with fura-2, was measured, and the results and the noise were subtracted from the results obtained in fura-2-loaded cells. This had no effect on the bandwidth of the measurements. A calibration of the fura-2 fluorescence signal was attempted at the end of each experiment using the Ca$^{2+}$ ionophore ionomycin (1 μM) and low and high Ca$^{2+}$ buffers. [Ca$^{2+}$] was calculated from the fluorescence ratio 340/380 nm according to the equation described by Grynkiewicz et al. (18).

**Expression of Prostanoid Receptor mRNA in Mouse Glomeruli and Podocytes**

The RNA preparation, the reverse transcription, and the PCR amplification (PCR) were performed according to the method recently described (19). In brief, total RNA from cultured mouse podocytes was isolated with guanidinium/acid phenol/chloroform extraction, and the amount of RNA was measured by spectrophotometry. For first strand synthesis, 2 μg of total RNA from podocytes was mixed in 5× reverse transcription buffer and incubated with 5 U of DNase I for 15 min. After termination, the reaction was completed with 0.5 mM dNTP, 0.5 μM sequence-specific primers, 10 mM dithiothreitol, and 200 U SuperScript II transcriptase. (Reverse transcriptase was omitted in some experiments to control for amplification of contaminating DNA.) The reverse transcription was performed at 42°C for 1 h, followed by a denaturation at 95°C for 5 min. cDNA was purified from amplification reaction and dissolved in 30 μl of 10 mM Tris buffer, pH 8. PCR was performed in duplicate in a total volume of 20 μl, each containing 2 μl of reverse transcription reaction and 18 μl of PCR master mixture containing 10 pmol each of sense and antisense primer and 2.5 U of Taq DNA polymerase. The cycle profile included denaturation for 30 s at 94°C, annealing for 30 s at 52°C, and extension for 30 s at 72°C. The amplification products of 10 μl of each PCR reaction were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized by ultraviolet irradiation.

The primers were:

**EP$_1$ receptor** [D16338]: f-5′-GCTGTACGCTGACATCGTG-3′

r-5′-CCTTAGCCACAGCCAAGATG-3′

**EP$_2$ receptor** [D50589]: f-5′-CTTGGCCTGCTCAACTAC-3′

r-5′-GTCTCCTCTGCCATGAACTTC-3′

**IP receptor** [D21024]: f-5′-GGCTGCTCTGGTGGTACCT-3′

r-5′-AACAGCCACATCAAGTATAGA-3′

**EP$_4$ receptor** [D13458]: f-5′-TGCTCATCGCTCCATTCGCAG-3′

r-5′-CTGCTGACTCTTAAACTCCC-3′

**FP receptor** [U47287]: f-5′-CTTCTTCTGCTTTTGTGCT-3′

r-5′-GGTTGAGCTGCTGATGTTG-3′

**TP receptor** [D10849]: f-5′-CCTTGGTCTCACCAGGCTCC-3′

r-5′-GCTGAAACATCTCCACCC-3′

**IP receptor** [D26157]: f-5′-TGCTGGCTTCCATCCCTAC-3′

r-5′-CAAACCAGAATTGGCGT-3′

The National Center for Biotechnology Information accession no. of the respective nucleotide sequence is given in brackets. PCR amplification of reverse transcription (RT) reactions without reverse transcriptase revealed no PCR product, thereby excluding amplification of genomic DNA. Identity of amplification products was determined by dyeoxy sequencing.

**Chemicals**

The following agents were used: prostaglandin E$_1$ (PGE$_1$), 11-deoxy-PGE$_1$, BW 245 C, sulprostone, AH 6809, SC 51089, U-46619, fluprostenol, (all from Cayman Chemical, Ann Arbor, MI), PGE$_2$, PGF$_{2α}$, forskolin (Sigma), iloprost (kindly donated by Schering, Ber-
lin, Germany), butaprost (kindly donated by Dr. Stünkel, Bayer AG, Leverkusen, Germany).

**Statistical Analyses**

The data are given as mean values ± SEM, where \( n \) refers to the number of experiments. The average of the effect of the agonist before and after the experiment was taken as control. Paired \( t \) test was used to compare mean values within one experimental series. A \( P \) value ≤0.05 was considered statistically significant.

**Results**

**Prostaglandins Depolarize the Membrane Voltage of Podocytes**

In 106 experiments in the slow whole cell configuration, a stable resting \( V_m \) of \(-59 ± 1 \text{ mV} \) was obtained for the control period. Addition of PGE\(_2\) caused a long-lasting and reversible depolarization of podocytes (\( n = 106 \)). PGE\(_2\) (100 nM) depolarized podocytes by \( 14 ± 1 \text{ mV} \) (\( n = 54 \)). During the depolarization, the conductances for the inward and outward current increased significantly from \( 2.0 ± 0.3 \) to \( 2.6 ± 0.3 \) nS and \( 1.7 ± 0.2 \) to \( 2.3 ± 0.3 \) nS, respectively (\( n = 50 \)). Figure 1 shows a typical recording of the effect of 100 nM PGE\(_2\) on \( V_m \) and \( G_m \) of a podocyte.

**Effect of Various Prostanoid Agonists on Membrane Voltage of Podocytes**

To characterize the prostanoid receptor involved in the response to PGE\(_2\), the \( V_m \) response to different prostaglandins and synthetic prostaglandin agonists was tested (20,21). Figure 2 shows the concentration response curves of the \( V_m \) response to PGE\(_1\), PGE\(_2\), 11-deoxy-PGE\(_1\) (EP\(_2\)/EP\(_3\)/EP\(_4\)-receptor agonist), BW 245 C (DP receptor agonist), and sulprostone (EP\(_1\)/EP\(_3\) receptor agonist). Compared with PGE\(_2\), PGE\(_1\) induced a slightly stronger depolarization. The depolarizing effect of PGE\(_2\) and 11-deoxy PGE\(_1\) were equipotent, whereas the DP receptor-selective agonist BW 245 C and the EP\(_1\)/EP\(_3\) receptor-selective agonist sulprostone were less potent than PGE\(_2\). One micromolar iloprost (prostacyclin agonist) depolarized podocytes by \( 5 ± 1 \text{ mV} \) (\( n = 6 \)), whereas 1 \( \mu \)M of the EP\(_2\) receptor agonist butaprost had no effect on membrane voltage of podocytes (\( n = 5 \)) (data not shown).

**Influence of a Reduced Extracellular Cl\(^-\) or Na\(^+\) Concentration on the Depolarization Induced by PGE\(_2\)**

Under resting conditions, PGE\(_2\) depolarized podocytes by \( 13 ± 1 \text{ mV} \). Reduction of the extracellular Cl\(^-\) concentration (from 147 to 32 mM) caused a slight hyperpolarization by \( 8 ± 1 \text{ mV} \). Paired experiments showed that the depolarizing effect of PGE\(_2\) in the presence of 32 mM Cl\(^-\) (100 nM) was significantly augmented from \( 13 ± 1 \) to \( 27 ± 3 \text{ mV} \) (six paired experiments) (Figure 3).

Next, we examined the effect of a removal of the extracellular Na\(^+\) on the \( V_m \) response to PGE\(_2\) in seven paired experiments. Under resting conditions, PGE\(_2\) depolarized podocytes by \( 13 ± 1 \text{ mV} \). When Na\(^+\) was removed from the extracellular bath solution (0 mM versus 145 mM Na\(^+\)), \( V_m \) hyperpolarized transiently by \( 4 ± 1 \text{ mV} \) (\( n = 7 \)). In the absence of extracel-

Figure 1. Typical recording of the effect of prostaglandin E\(_2\) (PGE\(_2\)) (100 nM) on membrane voltage (\( V_m \), top panel) and the whole cell conductance (\( G_m \), bottom panel) in podocytes. To obtain \( G_m \), the voltage of the respective cell was clamped to the resting \( V_m \). Starting at this value, the whole cell current was measured by depolarizing or hyperpolarizing \( V_m \) in steps of 10 mV to ±40 mV.

Prostaglandins Stimulate cAMP Accumulation in Podocytes

Because multiple PGE\(_2\) receptors exist that are coupled to different signaling pathways, we first investigated the effect of PGE\(_2\) on intracellular cAMP. Incubation of podocytes with 100 nM PGE\(_2\) resulted in a time-dependent accumulation of cAMP (Figure 4A). The effect of PGE\(_2\) on cAMP accumulation was concentration-dependent (Figure 4B).

Figure 5 shows the effect of different prostanoid agonists on cAMP increase in podocytes. Like PGE\(_2\), PGE\(_1\) (50 nM), lular Na\(^+\), the effect of PGE\(_2\) was not inhibited, i.e., PGE\(_2\) (100 nM) depolarized podocytes by \( 20 ± 2 \text{ mV} \) (data not shown).
11-deoxy-PGE₂ (500 nM), BW 245 C (10 µM), and sulprostone (10 µM) stimulated an increase of cAMP accumulation, whereas iloprost (1 µM) and butaprost (1 µM) did not increase cAMP in podocytes (n = 6 for each agonist).

**Effect of Prostanoids on [Ca²⁺]ᵢ in Podocytes**

In 113 of 185 experiments, addition of PGE₂ (10 nM to 10 µM) resulted in a reversible and concentration-dependent increase of [Ca²⁺]ᵢ in podocytes, whereas stimulation of cAMP production by 1 µM forskolin had no effect on [Ca²⁺]ᵢ (n = 5, data not shown). The [Ca²⁺]ᵢ increase induced by PGE₂ and all other tested prostanoids (see below) was biphasic with a transient [Ca²⁺]ᵢ peak, which was followed by a [Ca²⁺]ᵢ plateau. In these and the following experiments, cells that did not respond to PGE₂ or different prostanoids responded to other vasoactive agonists such as bradykinin (n = 58) or extracellular ATP (n = 49).

In 12 of 20 experiments, a [Ca²⁺]ᵢ increase was observed after the addition of 1 µM of the EP₁/EP₃ receptor agonist sulprostone (20). Figure 6A shows a fluorescence recording of the effect of PGE₂ and sulprostone on [Ca²⁺]ᵢ in podocytes, and Figure 6B summarizes the data.

To identify the EP receptor involved in the PGE₂-mediated [Ca²⁺]ᵢ response, experiments with the putative EP receptor antagonists SC 51089 and AH-6809 (20) were performed. SC 51089 itself increased [Ca²⁺]ᵢ in podocytes (change of the fluorescence ratio from 1.1 ± 0.1 to 1.4 ± 0.2 [1 µM, n = 8] and from 1.3 ± 0.2 to 2.0 ± 0.4 [10 µM, n = 6]). SC 51089 (1 or 10 µM) did not inhibit PGE₂-mediated [Ca²⁺]ᵢ response (data not shown). AH-6809 (100 µM) itself increased [Ca²⁺]ᵢ, in podocytes (change of the fluorescence ratio from 1.0 ± 0.1 to 1.5 ± 0.2, n = 4). In the continuous presence of 100 µM AH-6809, the [Ca²⁺]ᵢ response to 0.1 µM PGE₂ was completely inhibited. After removal of AH-6809 and rinsing cells with control solution, PGE₂ failed to increase [Ca²⁺]ᵢ, indicating that the effect of AH-6809 was irreversible (n = 4, data not shown). Lower concentrations of AH-6809 (10 µM) did not change resting [Ca²⁺]ᵢ. In the presence of 10 µM AH-6809, the [Ca²⁺]ᵢ response to 0.1 µM PGE₂ was significantly inhibited by 39 ± 14% (n = 10). After rinsing cells with control solution, a following [Ca²⁺]ᵢ response to PGE₂ was still reduced. Figure 7A shows an original experiment of the effect of PGE₂ on [Ca²⁺]ᵢ in the absence and presence of AH-6809, and Figure 7B summarizes the data.

PGE₂ (10 nM to 10 µM) increased reversibly and concentration-dependently [Ca²⁺]ᵢ, in podocytes in 81 of 109 experiments (Figure 8, A and B).

Figure 9A shows that U-46619, a selective thromboxane A₂ receptor agonist (20), reversibly increased [Ca²⁺]ᵢ, in a podocyte. This could be reproduced in 34 of 54 cells. Figure 9A also shows that in contrast to PGE₂ and fluprostenol, after removal of U-46619 and rinsing cells with a Ringer’s-like solution for up to 10 min, U-46619 did not evoke a second [Ca²⁺]ᵢ response (n = 5). In contrast, the addition of fluprostenol after a U-46619-mediated [Ca²⁺]ᵢ increase still led to a second [Ca²⁺]ᵢ response (n = 5). Figure 9B shows the concentration dependence of the effect of U-46619.

**Podocytes Express mRNA for the EP₁, EP₃, FP, and TP Receptor**

In another approach, we studied the expression of the EP receptor subtypes in mouse podocytes on the mRNA level.
RNA was analyzed by RT-PCR technique using sequence-specific primers to the different EP receptor subtypes. Figure 10 shows ethidium bromide-stained agarose gel electrophoresis of PCR products for the EP1 to EP4, FP, and TP receptor in mouse podocytes. Expression of β-actin was used as an internal standard. Sequence analysis of the resulting amplification products revealed the sequence identity for the fragments. An EP2 receptor could not be detected in cultured podocytes (lane 2). The faint band in the EP3 lane had no sequence homology to any of the EP receptors, indicating an amplification artifact. Even after 40 cycles, an IP receptor could not be detected in podocytes by RT-PCR. However, we detected the IP receptor in mouse aorta and mouse spleen, indicating that the primers chosen were able to amplify the IP receptor (data not shown).

Discussion
Prostaglandins are produced within the glomerulus and are known to modulate glomerular functions by acting locally on cells in which they are synthesized (22). Recently, we have shown that the prostaglandin-generating enzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) are primarily expressed in the podocyte of the human kidney (23). Very recently it has been demonstrated that COX-2 expression is exclusively enhanced in podocytes during the course of Thy-1 nephritis, suggesting that mesangial cell injury stimulates COX-2-induced prostaglandin synthesis in podocytes (24). The mechanism of proteinuria in Thy-1 nephritis is not clearly understood, but it may be speculated that the increase of prostanoid synthesis in podocytes might, in an autocrine manner, change their cellular functions leading to an impairment of podocyte barrier function. The present study demonstrates that prostaglandins regulate cellular functions of the podocyte via specific prostanoid receptors. Prostanoid receptor agonists depolarized podocytes and increased their whole cell conductance. The depolarization was probably mediated by an opening of a Cl⁻ conductance because the effect of PGE₂ on membrane voltage of podocytes was accompanied by an increase of the whole cell conductance, and PGE₂-induced depolarization was augmented in a solution with a reduced Cl⁻.

Figure 4. PGE₂ stimulates cAMP accumulation in podocytes. (A) Time dependence of cAMP increase induced by PGE₂ (100 nM). Mean values ± SEM. Podocytes were preincubated with 0.5 M 3-isobutyl-l-methylxanthine (IBMX) for 5 min and stimulated with PGE₂ for the indicated time. n = 3 for each data point. (B) Concentration–response curve of the cAMP increase induced by PGE₂ (n = 3 to 15).

Figure 5. Summary of the effect of iloprost (1 μM), butaprost (1 μM), sulprostone (10 μM), BW 245 C (10 μM), PGE₁ (50 nM), and 11-deoxy-PGE₁ (500 nM) on cAMP accumulation in podocytes (n = 6 for each data point). After preincubation with 0.5 M IBMX for 5 min, podocytes were exposed to the indicated agent and the assay was terminated after an incubation time of 5 min. n = 6 for each data point.
concentration. In contrast, the membrane voltage response to PGE$_2$ was unchanged in the absence of extracellular Na$^+$, indicating that it was not due to an influx of Na$^+$. A cAMP- and Ca$^{2+}$-dependent Cl$^-$ conductance has been detected recently in podocytes (25). Within the kidney, a PGE$_2$-mediated activation of Cl$^-$ channels has been demonstrated in principal cells (26), whereas up to now nothing is known about PGE$_2$-mediated regulation of Cl$^-$ channels in glomerular cells. The precise cellular role for the activation of the Cl$^-$ conductance in podocytes is unknown, but it may be speculated that the activation of the Cl$^-$ conductance by PGE$_2$ contributes to the cell volume regulation in these cells (27).

The prostanoid receptor family includes the so-called DP, EP, FP, IP, and TP receptors, which preferentially bind prostaglandin D (PGD), PGE, PGF, PGI, and thromboxane A (TXA), respectively. Moreover, four subtypes (EP$_1$ through EP$_4$) of the EP receptor have been characterized (20,22). To identify the prostanoid receptor involved in the depolarization induced by PGE$_2$, we examined the influence of synthetic prostaglandin analogs on the membrane voltage response in podocytes. Compared with PGE$_2$, 11-deoxy PGE$_1$, an EP$_2$/EP$_3$ receptor agonist, was equipotent, whereas the DP receptor agonists BW 245 C, the EP$_1$/EP$_3$ receptor agonist sulprostone, and the IP receptor agonist iloprost were much less potent than PGE$_2$. The selective EP$_2$ receptor agonist butaprost did not depolarize podocytes. This indicates that the depolarizing effect of PGE$_2$ was most likely mediated by an EP$_4$ receptor.

The suggested involvement of an EP$_4$ receptor in PGE$_2$ action was further corroborated by measurements of cAMP. 11-deoxy PGE$_1$, induced a marked stimulation of cAMP accumulation in podocytes, whereas butaprost had no effect. The existence of an EP$_4$ receptor in podocytes was further confirmed by the detection of EP$_4$ receptor mRNA in podocytes, whereas EP$_2$ receptor mRNA could not be detected. These data are in agreement with in situ hybridization studies by others.
which demonstrate that the EP₄ receptor is highly expressed in the mouse and human glomerulus (28,29). The existence of the EP₄ receptor in podocytes suggests that it might, at least in part, mediate the effects of PGE₂ on glomerular functions.

Micromolar concentrations of the DP receptor agonist BW 245 C (20) only had a weak effect on membrane voltage and cAMP generation in podocytes, indicating that the effects of PGE₂ are not mainly mediated via a DP receptor. Future studies need to clarify whether this receptor is expressed in podocytes at all.

In the present study, the IP receptor agonist iloprost has no effect on cAMP accumulation in podocytes, and an IP receptor could not be detected in RT-PCR studies, indicating that the effects of PGE₂ are not mainly mediated via a DP receptor. The slight depolarizing effect of iloprost may be explained by a small affinity of iloprost to other EP receptors (21). Very recently in kidney slices an IP receptor has been demonstrated in human podocytes (30). The lack of expression of the IP receptor in mouse podocytes may be explained by species differences. Alternatively, podocytes might lose the IP receptor under in vitro conditions.

In RT-PCR studies, a fragment for the EP₄ and for the EP₁ receptor could be amplified, whereas a fragment for the EP₃ receptor could not be detected. The possible existence of an EP₁ receptor in podocytes was surprising because mouse and human glomerular mRNA for the EP₁ receptor has not been detected by in situ hybridization (29,30). The lack of expression of the glomerular EP₁ receptor in the latter studies might be explained by the absence of EP₁ mRNA under physiologic conditions, i.e., an expression of the EP₁ receptor might occur only under pathologic or under in vitro conditions. Alternatively, it is possible that a low expression of EP₁ receptor mRNA in podocytes, which represent only a very small portion of the glomerular cell population, is hardly detectable by in situ hybridization. Recently, it has been reported that the mouse genes for the EP₁ receptor and the PKN protein kinase overlap (31). However, in this study the possibility that the EP₁ amplification products in podocytes represented PKN protein kinase mRNA expression has been excluded because, compared with the study by Båtshake et al., we used a different
approach of RT-PCR. Båtshake et al. used oligo-dT for reverse transcription and thereby transcribed both the mRNA species from sense and from antisense strand. The following PCR resulted in the fragments as described by Båtshake et al. (31). Instead of using this approach, we replaced oligo-dT by sequence-specific primers for reverse transcription, which transcribe only the mRNA corresponding to the sense strand but not the mRNA corresponding to the antisense strand. In addition, the RNA was DNase-treated to exclude the possibility of accidental amplification of contaminating genomic DNA.

The EP1 receptor is known to be coupled to phosphatidylinositol hydrolysis, resulting in an increase of [Ca$^{2+}$]$_i$ (20,22). To clarify the possible functional existence of an EP1 receptor in podocytes, measurements of [Ca$^{2+}$]$_i$ were performed in three different cloned podocyte cell lines. In some of the experiments, PGE$_2$ failed to increase [Ca$^{2+}$]$_i$ in podocytes, but in about 60% of the experiments, a weak, but concentration-dependent, biphasic [Ca$^{2+}$]$_i$ increase could be elicited by PGE$_2$. The results are in agreement with reports in mesangial cells, in which PGE$_2$ also induces a weak and inconsistent increase in [Ca$^{2+}$]$_i$ (32).

To further characterize the EP receptor involved in the PGE$_2$-mediated [Ca$^{2+}$]$_i$, response, experiments with the EP1/EP3 receptor agonist sulprostone and two different EP receptor antagonists, SC 51089 and AH-6809, were performed (20). Like PGE$_2$, sulprostone increased [Ca$^{2+}$]$_i$ in podocytes, indicating the existence of an EP1 or EP3 receptor. SC 51089 (1 and 10 µM) and higher concentrations of AH-6809 (100 µM) itself increased [Ca$^{2+}$]$_i$, suggesting that the EP1 receptor antagonists had an intrinsic receptor activity or induced some nonspecific cellular effects. AH-6809 (10 µM) did not influence [Ca$^{2+}$]$_i$ and slightly inhibited the [Ca$^{2+}$]$_i$ response to PGE$_2$. These results are in agreement with binding studies showing that SC 51089 and AH-6809 have a very low affinity to the EP1 receptor of the mouse, suggesting that there are species differences (21). Thus, although the EP1 antagonists had only small effects in this study, the [Ca$^{2+}$]$_i$ response to PGE$_2$ may be—also because of the lack of an EP3 receptor expression in RT-PCR experiments—mediated by an EP1 receptor.

It has been assumed that PGE$_2$ acts predominantly as a glomerular vasodilator, but the ability of PGE$_2$ to increase [Ca$^{2+}$]$_i$ in podocytes and mesangial cells (33) suggests an additional role for PGE$_2$ as a glomerular vasoconstrictor. We recently reported that the Cl$^-$ conductance of podocytes is regulated by cAMP and by [Ca$^{2+}$]$_i$ (25). Therefore, we cannot exclude that in some patch-clamp experiments the membrane voltage change induced by PGE$_2$ is partly due to an activation of a Ca$^{2+}$-dependent Cl$^-$ conductance.

Not only PGE$_2$, but also PGF$_{2\alpha}$, fluprostenol (a highly selective FP receptor agonist), and U-46619 (a TP receptor agonist) (20) increased [Ca$^{2+}$]$_i$ in podocytes. In contrast to PGE$_2$ and the FP agonists, repeated addition of U-46619 could not induce a second [Ca$^{2+}$]$_i$ response, suggesting that the TP receptor involved might be rapidly desensitized by the agonist. Compared with the FP agonists, the [Ca$^{2+}$]$_i$ response to U-46619 was about 100 times more potent and, after addition of U-46619 cells, still responded to fluprostenol, suggesting that the effect of fluprostenol and U-46619 was mediated by different receptors. In addition, the results of the RT-PCR studies indicate that both an FP and a TP receptor are expressed in podocytes. Little is known about the glomerular function of the FP receptor. It has been recently reported that the Cl$^-$ conductance of podocytes is regulated by cAMP and by [Ca$^{2+}$]$_i$ (25). Therefore, we cannot exclude that in some patch-clamp experiments the membrane voltage change induced by PGE$_2$ is partly due to an activation of a Ca$^{2+}$-dependent Cl$^-$ conductance.

Thromboxane A$_2$ has been suggested to regulate the ultrafiltration coefficient, and it is involved in a variety of glomerular diseases such as different forms of glomerulonephritis and diabetic nephropathy (34,35). In Heymann nephritis, an experimental model of membranous nephropathy in which the podocyte is the primary target cell of injury, inhibition of thromboxane A$_2$ synthesis prevented alterations of glomerular morphology and proteinuria (36). The TP receptor in podocytes might therefore be involved in the regulation of physio-
logic functions like the control of the ultrafiltration coefficient and might also play a role during podocyte injury.

The lack of a [Ca\(^{2+}\)] response toward the agonists in some of the cells suggests that the expression of the receptor proteins is low. Alternatively, the signaling system activated by PGE\(_2\), PGF2\(\alpha\), and thromboxane A\(_2\) via their receptors may be inhibited in some cells by a yet unknown mechanism. Future studies are needed to clarify the reasons for the unstable functional expression of the [Ca\(^{2+}\)], mobilizing receptors.

In conclusion, the data indicate that prostanoids regulate important cellular functions of podocytes via different prostanoid receptors: They depolarize podocytes, increase their cAMP, and stimulate [Ca\(^{2+}\)], in a part of podocytes. The coexpression of the prostaglandin receptors might balance opposite cellular effects of PGE\(_2\) in podocytes. The activation of the prostaglandin receptors in podocytes might influence glomerular hemodynamics, and it might be an important event in the pathogenesis of proteinuria.

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**References**


