Temporal expression of the PGE\textsubscript{2} synthetic system in the kidney is associated with the time frame of renal developmental vulnerability to cyclooxygenase-2 inhibition

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Frölich S, Olliges A, Kern N, Schreiber Y, Narumiya S, Nüsing RM. Temporal expression of the PGE\textsubscript{2} synthetic system in the kidney is associated with the time frame of renal developmental vulnerability to cyclooxygenase-2 inhibition. Am J Physiol Renal Physiol 303: F209–F219, 2012. First published May 9, 2012: doi:10.1152/ajprenal.00418.2011.—Pharmacological blockade of the cyclooxygenase (COX) pathway of arachidonic acid in renal tissue is associated with the time frame of renal developmental vulnerability to cyclooxygenase-2 (COX-2) inhibition. We investigated the temporal expression of the COX-derived PGE\textsubscript{2} system in isolated glomeruli and in renal cortex and medulla of postnatal neonatal mice. A reduced number of superficial glomeruli was observed by the 10th day of life, and the growth of the renal parenchyma was significantly diminished during this time frame in the COX-2-deficient mouse. COX-2 inhibition of the mPGES-1 enzyme was found closely related to the temporal expression of renal developmental damage. COX-2 inhibition of mPGES-1 was demonstrated to postnatal COX-2 activity is necessary for normal nephrogenesis and development of the renal cortex. Nephrogenesis in the kidney begins at embryonic day 11 and ends ~10–14 days postpartum (P10–P14). During this period, new nephrons are continually being generated from the nephrogenic mesenchyme in response to signals emanating from the tips of the branching ureter (7). The kidney continues to develop from the center toward the periphery, and the most mature nephrons are located in the inner cortex, the least mature in the outer cortex. In COX-2–/– mice, kidneys appear neonatally normal, but later kidney development is impaired demonstrated by hypoplastic glomeruli, a thinned cortical area, and immature glomeruli. A reduced number of glomeruli, appearance of undifferentiated cells, and frequently medullary hyperplasia have also been observed in COX-2–/– mice (8, 29), in addition to periglomerular and diffuse interstitial fibrosis (32). A recent study has shown that in mouse kidneys COX-2 protein is detectable at P4 and disappears thereafter (23). However, it is unknown whether this time point represents the vulnerable time window for use of COX-2 inhibitors. Comparable to mice, rat kidney nephrogenesis also lasts until ~P14 (28). However, in contrast to mice, maximal expression of COX-2 protein has variably been observed on days P7 (38), P14 (47), or P15 (44), which then declined until adulthood.

Although these observations gave clear evidence that COX-2 expression and activity are important prerequisites to ensure normal renal development in mice, the involved secondary prostanoid system and the exact time window for COX-2 necessity are not clarified in detail. COX is the rate-limiting step, but secondary PGH\textsubscript{2}-metabolizing enzymes are necessary to form specific prostanoids, and the kidney is able to produce all primary prostanoids, PGE\textsubscript{2}, PGI\textsubscript{2}, PGD\textsubscript{2}, PGF\textsubscript{2\alpha}, and thromboxane A\textsubscript{2} (for a review, see Ref. 37), whereas an important physiological function has been attributed to PGE\textsubscript{2}. In support of a developmental role of PGE\textsubscript{2} in isolated glomeruli from 10-day-old rats, PGE\textsubscript{2} was found as a major cyclooxygenase product (3), and we observed PGE\textsubscript{2} as the major prostanoid in urine samples of P4 mouse pups (unpublished observations, Frölich S). Three different enzymes are reported to be able to catalyze the synthesis of PGE\textsubscript{2} from COX-derived prostaglandins: PGE synthase; NSAID; nephrogenesis.
PGH2: microsomal PGE synthase (mPGES)-1, mPGES-2, and
cytosolic PGE synthase (cPGES), which are structurally and
biologically distinct enzymes (for a review, see Refs. 12 and 24).
mPGES-1 is markedly induced by proinflammatory stimuli and
downregulated by anti-inflammatory glucocorticoids as in the
case of COX-2. According to a recent report, it is functionally
coupled with COX-2 in marked preference to COX-1 (31).
mPGES-2 is rather constitutively expressed in various cells and
tissues and is thought to be functionally coupled to both
COX and COX-2 (30). cPGES is constitutively expressed in
a wide variety of cells, and evidence has been presented for
functional association of cPGES and COX-1 to promote im-
mediate PGE2 production (39). However, the relevance of
cPGES to the generation of PGE2 in vivo has been questioned
(26). Distinct renal functions for the three PGE synthases have
not yet been identified, but emerging evidence suggests a
potential role for mPGES-1 in the proximal tubular response in
aldosterone escape (17) and in the regulation of sodium ho-
moestasis (9, 18).

In the present paper, we questioned whether the PGE2
system may be involved in COX-2-dependent regulation of
renal development and whether vulnerability to COX-2 inhibi-
tion is associated with the PGE2 biosynthetic system in a
timely fashion. We hypothesize that, next to the COX-2 step in
renal arachidonic acid cascade, the PGE synthase step is also
subject to control during nephrogenesis. Therefore, we studied
time-dependent postnatal renal expression of mPGES-1,
mPGES-2, and cPGES, PGE2 synthetic activity, and also
urinary PGE2 level in mice. With a view to associate altera-
tions in the PGE2 system to changes in the COX system, we
also analyzed expression of COX-1 and COX-2 and the time
frame of renal developmental sensitivity toward COX-2 inhibi-
tion. Addressing the issue will help in understanding the
physiological role of the prostanooid system in renal develop-
ment.

MATERIALS AND METHODS

Animals and treatment. All animal care and experimental proce-
dures were in accordance with institutional guidelines and approved
by the Animal Welfare Committee of the State Agency (Darmstadt,
Germany). Breeder pairs of C57BL/6j wild-type mice were obtained
from Jackson Laboratories (Bar Harbor, ME). Breeder pairs of COX-
2-/- mice were kindly provided by Robert Langenbach (National
Institute of Environmental Health Sciences, Research Triangle Park,
NC) (29). EP1-, EP2-, EP3-, and EP4-deficient mice were generated
as previously described (15, 36, 42, 46). All mice are backcrossed to
C57BL/6 more than 10 times. Mice of each gender were used in the
study. Genotypes of the mice were determined by PCR analysis using
primers detected protein bands were visualized by the ECL detection
method according to the manufacturer’s protocol (Pierce, Rockford,
IL) using BSA as a protein standard. Samples of 50 μg protein were
mixed with 4× Laemmli sample buffer (0.1 mol/l Tris-HCl, 8% SDS,
40% glycerol, 0.1% bromophenol blue, 10% β-mercaptoethanol, pH
6.8) and heated for 10 min at 95°C. The samples were separated on
15% polyacrylamide minigels and transferred to nitrocellulose mem-
branes (Protran, Whatmann, Dassel, Germany). The membranes were
blocked with 5% nonfat dry milk in phosphate-buffered saline and
incubated with the primary antibodies. The following antibodies were
used: anti-COX-1 and anti-COX-2 (Cayman Chemical, Ann Arbor,
MI) diluted 1:150, anti-mPGES-1 (AgriSera, Vännäs, Sweden) diluted
1:5,000, anti-mPGES-2 (Cayman Chemical) diluted 1:2,000, and
anti-cPGES (Cayman Chemical) diluted 1: 50 in 5% nonfat dry milk in
PBS containing 0.1% Tween 20 overnight at 4°C. After washing
three times, membranes were incubated with secondary horseradish
peroxidase-labeled antibody diluted 1:5,000 in 5% nonfat dry milk in
PBS containing 0.1% Tween 20 for 1 h at room temperature. Immu-
nodetected protein bands were visualized by the ECL detection
system according to the instruction of the supplier (GE Healthcare,
Freiburg, Germany).

Quantitative real-time PCR. Kidneys from mice were dissected and
stored at −80°C until analysis. Total RNA was isolated using an
RNeasy kit (Qiagen, Hilden, Germany) according to the manufactur-
er’s instructions. The RNA concentration was quantified by A260 using
a NanoDrop spectrophotometer (Peglab Biotechnologie, Erlangen,
Germany). RNA (0.5 μg) was reverse transcribed using superscript II
RNase and random hexamers (Invitrogen, Karlsruhe, Germany), and
1/40 volume of the resulting cDNA samples were used as templates
for real-time PCR by using the SYBR Green supermix reaction
procedure with the 7500 Fast System (Applied Biosystems, Darm-
stadt, Germany). All reactions were run in triplicate to minimize
experimental error. The following primers pairs were used: for
COX-1, GTG GCT ATT TCC TGC AGC TC and CAG TGC CTC

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Kidney sections were stained specifically. The results are given as stained area of basement membrane with a numeric camera connected to the microscope. Results are presented as positive cells/field.

**Statistics.** Data are presented as means ± SE. Analysis were performed by ANOVA and a post hoc Bonferroni’s multiple comparison test for all groups or Dunnnett’s multiple comparison test vs. the control group. Prism 5.0 software (GraphPad) was used for all calculations. A P value <0.05 was considered significant.

**RESULTS**

Postnatal renal expression of COX-1 and COX-2. For COX-1, we observed no significant changes in renal expression, neither in mRNA nor in protein expression at the time points chosen (Fig. 1, A and B). In contrast, postnatal expression of COX-2 was regulated time dependently (Fig. 1, C and D). Semiquantitative real-time PCR analysis revealed a significant rise in mRNA expression for COX-2 at P4 and P6, with a continuous decrease until P10, at which mRNA expression was similar to P21 (Fig. 1C). COX-2 protein expression followed in a similar slightly time-shifted manner, with an expression peak reached on P6 (Fig. 1D) lasting until P10. During this time course, COX-2 protein expression was increased about four- to fivefold compared with P2. On P21, COX-2 protein displayed a similar level of expression as on P2.

**PGE2 synthetic activity in the kidney.** To investigate whether changes in renal COX-2 expression were paralleled by changes in renal PGE2 synthetic activity, we determined enzymatic capacity of kidney tissue homogenates to synthesize PGE2 from exogenously added arachidonic acid. Compared with P2, significantly elevated PGE2-synthesizing activities were observed from P4 until P10 (Fig. 2A). The formation of PGE2 could be blocked by the addition of 10 μM indomethacin, indicating COX-dependent PGE2 synthesis. Whereas addition of the COX-1 inhibitor SC-560 to kidney homogenates prepared from 8-day-old mice has had only minimal effect on enzymatic activity, the addition of SC-236 or rofecoxib significantly suppressed PGE2 synthetic activity, indicating the importance of COX-2 to renal PGE2 synthesis (Fig. 2A). From P2 to P21, we collected spontaneous urine samples and determined excreted PGE2 amounts. Compared with P2, urinary PGE2 concentration was significantly elevated on P4, P6, P8, and P10, but not on P21 (Fig. 2B). Compared with the vehicle-treated control group, treatment of wild-type mice with the COX-2-selective inhibitor SC-236 significantly reduced postnatal renal excretion of PGE2 at any time point (Fig. 2B).

In accordance, treatment of wild-type mice with rofecoxib also decreased PGE2 excretion (shown for P8, Fig. 2B), whereas application of the COX-1 inhibitor SC-560 caused a minor, nonsignificant decrease in PGE2 excretion. To clarify whether induction of COX-2 is sufficient for elevated PGE2 synthesis or whether secondary PGE synthesis are concomitantly induced, we also studied PGE2 formation by kidney homogenates using PGH2 as a substrate. Compared with P2, a significant rise in enzymatic activity was observed from P4 to P8 (Fig. 2C), but not on P10. Elevated PGE2 synthase activity was also detected in kidneys from 21-day-old mice.

**Postnatal renal expression of mPGES-1, mPGES-2, and cPGES.** To elucidate the role of specific PGE synthases for renal PGE2 synthesis, we studied the postnatal expression of
mPGES-1, mPGES-2, and cPGES. For mPGES-1, significantly elevated mRNA expression was detected on P6 and P21 (Fig. 3A). In line with this observation, increased mPGES-1 protein expression was observed (Fig. 3B), which reached significance on P4, P6, and P21 compared with P2. In striking contrast to mPGES-1, renal mRNA and protein expression of mPGES-2 and cPGES remained unchanged at the indicated postnatal time points (Fig. 3, C and D and E and F, respectively).

Size of glomeruli in EP receptor-deficient mice. To further substantiate the developmental role of PGE2, we studied nephrogenesis in PGE2 receptor-deficient mice. Determination of glomerular size on P21 revealed that minor but significantly impaired growth of glomeruli was detectable in EP1−/−, EP2−/−, and in EP4−/− mice, whereas the growth of glomeruli in EP3 mice was not different from control (Fig. 4).

Time-dependent effects of COX-2 inhibition on nephrogenesis. To determine whether the transient expression and activity of the PGE2 system fall into the time frame of vulnerability toward COX-2 inhibition, we treated C57BL/6J wild-type mice with the selective COX-2 inhibitor SC-236 for different time courses. Administration of SC-236 from P1 to P2 already compromised the size of glomeruli, and we observed a significant reduction in mean diameter (control: 48.35 ± 0.97 μm; P1–P2: 41.53 ± 0.93 μm; P < 0.05) (Fig. 5, A and B). Prolonged exposure to SC-236 further downsized glomeruli, with a maximal effect following administration until P10. Additional administration of the COX-2 inhibitor did not further diminish glomerular diameter, and the observed size reduction was quite similar to COX-2−/− mice. Glomeruli from mice treated from P11 or P13–P20 were indistinguishable in mean diameter to control (Fig. 5, A and B).

In control kidneys, a mean subcapsular cortical thickness of 56.84 ± 1.24 μm was observed. COX-2 inhibition P1 and P2 already caused a reduction by ~36% to 36.33 ± 2.12 μm (Fig. 5C). The strongest cortical thinning was observed following administration of SC-236 from P1 to P8, which was significantly different to shorter times of exposure and comparable to the cortical defect in COX-2−/− mice. However, from this time point on no further reduction in subcapsular cortical thickness could be induced. Of note, administration of SC-236 starting on P11 and on P13 also caused a significant attenuation of cortical growth, albeit to a lesser extent (Fig. 5C).

Regarding the relative number of superficial glomeruli, we observed no significant difference from the control group following administration of SC-236 until P4 (Fig. 6A). Longer exposure of kidneys to the COX-2 inhibitor caused a significant increase in superficial glomeruli, which reached a maximum at P8. The effect in the group at P1–P10 was slightly stronger, but the difference to the group at P1–P8 was not significant. However, the observed changes were less dramatic as in COX-2−/− mice. Administration of SC-236 from P11 or P13–P20 was without an effect on the relative number of superficial glomeruli (Fig. 6A). Histomorphological damage was not limited to SC-236-inhibited COX-2, as we observed similar changes in glomerular diameter and subcapsular thickness by application of rofecoxib (Fig. 6, B and C). In accordance to previous reports (23, 33), we observed no effect of the COX-1 inhibitor SC-560 on glomerular or cortical growth (Fig. 6, B and C).

Time-dependent effects of COX-2 inhibition on inflammatory responses, cell differentiation, and fibrosis. In addition, we questioned whether different time courses of SC-236 admini-
tration would affect inflammatory responses, loss of cell differentiation, and fibrotic processes, reported to occur in COX-2/H11002/H11002 mice (32). We studied the number of interstitial macrophages by F4/80 staining used as an indicator of inflammatory response. Following administration of SC-236 for different time courses, only faint amounts in number of F4/80-positive cells were observed in all study groups (Fig. 7A), indicating no significant effect of COX-2 inhibition on macrophage migration into renal tissue. Next, we evaluated cortical renal cell proliferation as a measure of undifferentiated cells by immune staining for PCNA. Only a very small number of PCNA-positive cells were found in cortical sections of control kidneys on P21 (1.45 ± 0.25 cells/index field), indicating that differentiation processes within the kidney are nearly finished at this time point (Fig. 7B). For example, the number of PCNA-positive cells on P2 reaches 21.25 ± 1.76 cells/index field.

Fig. 2. Renal PGE2 synthetic activity and urinary PGE2 excretion. Kidneys were prepared at the indicated postnatal time points (P2, P4, P6, P8, P10, and P21), and protein homogenates were prepared. A: PGE2 synthetic activity was studied using 100 μg of kidney homogenate incubated with 50 μM arachidonic acid. Protein samples prepared on P8 were also analyzed in the presence of 10 μM SC-236, 10 μM rofecoxib, and 10 μM SC-560 (shown by white bars). Incubation in the presence of 10 μM indomethacin served as a control for COX dependent PGE2 synthesis (shown by open bar). Following extraction, PGE2 was determined by liquid chromatography tandem mass spectrometry analysis. Values are means ± SE of 3 independent experiments. *P < 0.05 vs. P2. #P < 0.05 vs. P8. $P < 0.05 vs. P8+SC-560. §P < 0.05 vs. P21. B: urinary PGE2 concentrations were determined by LC-MS/MS. Mice were treated for the indicated time points with vehicle (shown by open bars), SC-236, rofecoxib, or SC-560 (each at 10 mg·kg−1·day−1). Values are means ± SE of 4 independent experiments. *P < 0.05 vs. untreated group. #P < 0.05 vs. P2. C: PGE2 synthase activity was studied using 1 mg of kidney homogenate incubated with 10 μM PGH2. Values are means ± SE of 4 independent experiments. *P < 0.05 vs. P2.
Following administration of SC-236 for different time frames, we detected a significant increase in PCNA-positive cell number compared with control only at P1–P10 and P1–P20 (Fig. 7B). This finding indicates that a prolonged time of suppression of COX-2 activity is necessary to cause effects on renal cell differentiation. Periglomerular fibrosis was studied in Sirius red-stained kidney sections. Only continuous administration of COX-2 inhibitor SC-236 from P1 to P20 was able to raise the mean area of the basement membrane of Bowman’s capsules significantly (Fig. 7C).

**DISCUSSION**

The COX pathway of arachidonic acid is responsible for the synthesis of various prostanooids. In preliminary experiments, we identified PGE2 as a major prostaglandin formed during the first postnatal days in mice. Therefore, we questioned, whether a time frame of susceptibility toward COX-2 inhibitors is associated with changes in PGE2-synthesizing capabilities. We observed that COX-2 expression is upregulated within the first postnatal days, with maximal expression of COX-2 between P4 and P10, shown by an increase in mRNA and protein expression as well as enzymatic COX activity demonstrated by enhanced arachidonic acid turnover to PGE2. COX-1 expression remained unchanged. Similarly, peak expression of COX-2 protein on P4 in mouse kidney has been reported (23). Albeit COX-1 enzymatic activity was not studied, we assume that the increase in our COX activity assay and in renal PGE2 excretion is mainly attributed to the increase in COX-2 expression. Although PGE2 levels were determined in spot urine and may be influenced by hydration status or development of the concentrating ability of mice pups, a substantial decrease in PGE2 excretion following SC-236 or rofecoxib administration is in line with our conclusion that in mouse kidney the COX-2 enzyme is responsible for altera-

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tion in PGE₂ production within the first 10 days of life. In adult mice, it is suggested that mainly COX-1 contributes to renal PGE₂ formation under physiological conditions, whereas COX-2 activity is important under pathological conditions such as volume depletion, e.g., caused by long-term furosemide application (11). The importance of PGE₂ for normal renal development is supported by our observation that distinct EP receptor-deficient mice also exhibit impaired nephrogenesis. The diminished diameter of the glomeruli in EP₁⁻/⁻, EP₂⁻/⁻, and EP₄⁻/⁻ mice indicates a complex interaction of various EP receptors with glomerulogenesis. In support of our observation, a recent report has shown that in rat kidney EP₁, EP₂, and EP₄ receptor mRNA and protein increased after birth and then decreased to adult levels, whereas EP₃ receptor did not rise before 21 days of age (4). Further studies will be necessary to elucidate the specific developmental roles of the distinct EP receptor types.

Our observation that biosynthetic formation of PGE₂ from PGH₂ is also transiently induced let us suggest that PGE₂ synthases secondary to COX-2 are regulated in a comparable way to COX-2. No data are available regarding early postnatal expression of PGE synthases. We observed a similar transient renal induction of mPGES-1 as for COX-2 in early postnatal life. In contrast, mPGES-2 and cPGES expression was unchanged, at least during the time points investigated. A simultaneous rise in expression of COX-2 and mPGES-1 fits quite well with the assumption that mPGES-1 is colocalized and coregulated with COX-2 to act in concert to mediate PGE₂ synthesis (31).

Fig. 4. Diameter of glomeruli in EP-deficient mice. Kidneys were removed on P21, fixed in 4% paraformaldehyde (PFA), and histomorphometrically analyzed for diameter of glomeruli. Data for wild-type mice (control) are shown by the open bar. Values are means ± SE of 6 independent experiments. *p < 0.05 vs. control.

Fig. 5. Time-dependent effect of COX-2 inhibition by SC-236 on postnatal kidney development. SC-236 was administrated for the indicated time courses: from P1 to P2, from P1 to P4, from P1 to P10, from P1 to P20, from P11 to P20, and from P13 to P20 (the latter 2 shown by grey bars). Vehicle-treated wild-type C57BL6 mice served as a control, and untreated COX-2⁻/⁻ mice were used to demonstrate a maximal effect (shown by open bars). Kidneys were removed on P21, fixed in 4% PFA, and histomorphometrically analyzed. A: representative renal histology following SC-236 application for the indicated time frames (large arrow marks subcapsular cortical thickness; small arrow marks glomeruli). B: diameter of glomeruli. C: subcapsular cortical thickness. Values are means ± SE of 6 independent experiments. *p < 0.05 vs. control. #p < 0.05 vs. P1–P8, P1–P10, and P1–P20.
An outstanding role of mPGES-1 in adult renal COX-2-dependent PGE2 synthesis is also assumed. In mPGES-1-deficient mice, Francois et al. (9) observed an attenuation of COX-2-dependent, furosemide-induced urinary PGE2 excretion. Furthermore, immunological studies have shown that in mice and rat kidney COX-2 and mPGES-1 are expressed colocalized in the macula densa and thick ascending limb of Henle, whereas mPGES-1 and COX-1 were expressed in the distal tubule and in the collecting duct (6). Concerning transient temporal expression of COX-2 and mPGES-1 around P4–P8 and unaltered expression of COX-1, mPGES-2, and cPGES during this time frame, we hypothesize that concomitant induction of COX-2 and mPGES-1 by an as yet unidentified signal is necessary to deliver PGE2 for normal postnatal development and maturation of the kidney. mPGES-2 is constitutively expressed in several tissues, and its level of expression is unaltered under inflammatory conditions and it utilizes PGH2 deriving from COX-1 or COX-2 (30). In contrast, cPGES has been proposed to functionally couple predomi-

Fig. 6. Time-dependent effect of COX-2 inhibition on postnatal kidney development. COX inhibitors were administered for the indicated time courses. Vehicle-treated wild-type C57BL6 mice served as a control, and untreated COX-2−/− mice were used to demonstrate a maximal effect (shown by open bars). Kidneys were removed on P21, fixed in 4% PFA, and histomorphometrically analyzed. A: relative number of superficial glomeruli following administration of SC-236; B: diameter of glomeruli; C: subcapsular cortical thickness following administration of rofecoxib and SC-560 in the period P1–P8. Values are means ± SE of 6 independent experiments. *P < 0.05 vs. control. #P < 0.05 vs. P1–P2 and P1–P4.

Fig. 7. Time-dependent effect of COX-2 inhibition by SC-236 on relative number of cortical macrophages, proliferating cells, and fibrosis. SC-236 was administered for the indicated time courses. Vehicle-treated wild-type C57BL6 mice served as a control (shown by open bar). Kidneys were removed on P21. A: macrophages were detected by F4/80-positive staining. No significant differences among experimental groups were observed. B: proliferating cells were identified using an anti-proliferating cell nuclear antigen (PCNA) antibody. C: fibrosis was investigated by determination of area of basement membrane of Bowman’s capsule relative to glomerular diameter. Values are means ± SE of 3 independent experiments. *P < 0.05 vs. control.
that spinal cord segments from mPGES-1 nephrogenesis in COX-2 expression, mPGES-1 PGE2 as necessary during the postnatal phase of nephrogenesis, mPGES-1 expression. However, other sources, such as mPGES-2 or cPGES, may deliver necessary PGE2 and thereby compensate for mPGES-1 deficiency. Second, shunt of arachidonic acid to other prostanoids or eicosanoids may replace for missing PGE2. This assumption is supported by our recent observation that spinal cord segments from mPGES-1 deficiency. Moreover, the RAAS is hypothesized that regulation of COX-2 and mPGES-1 expression is a prerequisite for normal kidney development and maturation, less clear are the triggers for enzyme induction and the physiological processes actuated by PGE2. Evidence is given that expression of both COX-2 and mPGES-1 could completely be blocked by glucocorticoids, and it may be hypothesized that regulation of COX-2 and mPGES-1 expression in the developing kidney involves glucocorticoids. In rodents, glucocorticoid levels decrease precipitously at parturition and remain very low (14). On the other hand, postnatal supplementation with glucocorticoids suppressed renal COX-2 in rats (27, 47). We speculate that the drop in ambient glucocorticoid levels promotes the rise in renal COX-2 and mPGES-1 expression. Regarding the developmental role of PGE2, possibly an interaction with the renin-angiotensin-aldosterone system (RAAS) does exist. In rats, COX-2 contributes to elevated renin in the early postnatal period (38), and in the adult kidney PGE2 is able to activate RAAS via EP2 and EP4 receptors (10, 35). Next to EP1, both receptor types were identified by us to be involved in nephrogenesis in mice. Moreover, the RAAS is known to play a role in kidney development, and genetic or pharmacological knockout of the RAAS also causes renal developmental defects (16). Further studies will be necessary to identify the signaling mediators involved next to prostaglandins.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS
Author contributions: S.F., A.O., N.K., and Y.S. performed experiments; S.F., A.O., N.K., Y.S., and R.M.N. analyzed data; S.F., S.N., and R.M.N.
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