Localization of prostaglandin E2 EP2 and EP4 receptors in the rat kidney

BOYE L. JENSEN, JANE STUBBE, PERNILLE B. HANSEN, DITTE ANDREASEN, AND OLE SKØTT

Department of Physiology and Pharmacology, University of Southern Denmark-Odense, DK-5000 Odense C, Denmark

Received 28 June 2000; accepted in final form 24 January 2001

Jensen, Boye L., Jane Stubbe, Pernille B. Hansen, Ditte Andreassen, and Ole Skott. Localization of prostaglandin E2 EP2 and EP4 receptors in the rat kidney. Am J Physiol Renal Physiol 280: F1001–F1009, 2001.—We investigated the localization of cAMP-coupled prostaglandin E2 EP2 and EP4 receptor expression in the rat kidney. EP2 mRNA was restricted to the outer and inner medulla in rat kidney, as determined by RNase protection assay. RT-PCR analysis of microdissected resistance vessels and nephron segments showed EP2 expression in descending thin limb of Henle’s loop (DTL) and in vasa recta of the outer medulla. The EP4 receptor was expressed in distal convoluted tubule (DCT) and cortical collecting duct (CCD) in preglomerular vessels, and in outer medullary vasa recta. Butaprost, an EP2 receptor-selective agonist, dose dependently raised cAMP levels in microdissected DTL and outer medullary vasa recta specimens but had no effect in EP2-negative outer medullary collecting duct segments. Dietary salt intake did not alter EP2 expression in the kidney medulla. These results suggest that PGE2 may act in the resistance vessels and in the DTL and DCT-CCD segments as a paracrine, cAMP-dependent regulator of vascular resistance and tubular transport, respectively.

PROSTANOIDS HAVE IMPORTANT regulatory functions in the kidney. Renal tubular epithelium and interstitial cells synthesize and release predominantly PGE2 in a regulated fashion. PGE2 affects urine concentration ability, promotes salt and water excretion by the renal medulla, maintains renal blood flow and glomerular filtration rate (GFR), particularly under neurohumoral activation, and stimulates renin release (for a recent review, see Ref. 3). PGE2 initiates and maintains these physiological effects through interaction with specific receptors on target cell surface membranes. At least four different PGE2 receptor isoforms exist, designated EP1–4, with various intracellular coupling mechanisms (2, 6, 12, 18, 19, 23, 28, 35). In the kidney, all four known EP receptors are expressed at variable levels (4, 11, 17, 27). The natriuretic and diuretic effects of PGE2 are probably accomplished through interaction with phosphoinositol-coupled EP1 and Gi-coupled EP3 receptors situated on the ascending part of the loop of Henle and the collecting duct system (3, 4, 18, 27). By activation of separate EP1 and EP3 receptors in the collecting ducts, PGE2 inhibits arginine vasopressin (AVP)-stimulated water and sodium transport (10). PGE2 also has the ability to raise cAMP production, at least in distal convoluted tubules (DCT) and cortical collecting duct (CCD) segments (7, 22, 26, 31), leading to an increase in water permeability in CCD (22). These findings suggest the presence of cAMP-coupled EP2 and/or EP4 receptors for PGE2 at specific nephron sites in the kidney cortex. Moreover, both EP2 and EP4 receptors are expressed in the medulla (4, 11, 17), but the exact localization and function are not known. Renal EP2 receptors could play a role in renal salt and water handling, because mice deficient in functional EP2 receptor genes develop salt-sensitive hypertension (13, 30). The mechanism behind the development of hypertension in this model is not clear, but the lack of EP2 receptors in the kidney is a relevant possibility. In contrast to EP1 and EP3 receptors, little is known about the expression and functional role of EP2 and EP4 receptors along the nephron. It therefore appeared relevant to us to determine whether these receptors are expressed along the nephron and, if so, to determine whether changes in expression are involved in renal adaptation to dietary salt intake.

The action of PGE2 on renal blood flow is believed to be direct, through cAMP-coupled vascular receptors, because PGE2 elicits vasodilation in isolated afferent arterioles and in descending vasa recta (8, 20). Recent data have confirmed the preglomerular expression of vasodilator EP receptors (17, 21, 29). However, it is unknown whether EP receptors are present in postglomerular resistance vessels, in particular vasa recta, which are highly sensitive to PGE2 (15, 20, 25). A second purpose of the present study was to investigate renal vascular cAMP-coupled EP receptor expression. To address these issues, we applied RT-PCR analysis.
to localize EP2 and EP4 receptors in microdissected nephron and vessel samples. Radioimmunoassay measurement of cAMP levels was applied after receptor-specific agonist stimulation.

METHODS

In vivo protocols. All procedures conformed with the Danish national guidelines for the care and handling of animals and with the published guidelines from the National Institutes of Health. Male Sprague-Dawley rats (150–220 g) had free access to standard pathogen-free rat chow (Na: 2 g/kg, Cl: 5 g/kg, Altromin-1310, Lage, Germany) and tap water. In one series, rats were kept on a high-salt diet (4% Na wt/wt; n = 6), and another group of rats received a NaCl-deficient diet (Altromin, 0.02% Na wt/wt; n = 6) for 10 days. The low-salt diet (Altromin-1036) contained 150 mg Na+/kg and 3.4 mg Cl−/kg. In the low-salt series, the rats were initially given an intraperitoneal injection of furosemide (2 mg/kg). The animals were killed by decapitation, trunk blood was sampled in EDTA-coated vials, and organs were rapidly removed, weighed, frozen in liquid nitrogen, and stored at −80°C. Kidneys were separated into major regions by dissection under a stereomicroscope and then snap-frozen in liquid nitrogen. The medullary rays were contained in the cortical tissue.

Extraction of RNA. Total RNA was extracted from tissue samples, basically according to the acid-guanidinium-phenol-chloroform extraction protocol of Chomczynski and Sacchi (5). RNA pellets were dissolved in diethylpyrocarbonate-chloroform extraction protocol of Chomczynski and Sacchi (5). RNA pellets were dissolved in diethylpyrocarbonate-chloroform extraction protocol of Chomczynski and Sacchi (5). The cloned DNA fragments were excised from the plasmids, and 50 ng were denatured for 2 min. The following components were added [final concentrations (in mM)]: 4 dATP, dGTP, and dTTP, respectively; 50 Tris-HCl, pH 8.0; 5 MgCl2, pH 8.0; 1 dithiothreitol; and 200 HEPES, pH 6.6, as well as 0.5 pmol/μl of each specific primer, 0.4 μg/μl BSA, 1 μCi/μl [α-32P]dCTP (Amersham Pharmacia Biotech, Birkerød, Denmark), and 0.05 U/μl Klenow to a final volume of 50 μl. Synthesis was continued for 2 h at room temperature. After denaturation at 95°C for 2 min, the probe was used. The agarose gels were equilibrated for 20 min in 1 liter of water followed by 2 × 10 min in NaOH (0.4 mol/l). DNA was transferred by capillary blotting to Zeta Probe GT membranes (Bio-Rad, Copenhagen, Denmark) for at least 2 h, the membrane was washed in 2× standard sodium citrate (SSC), air-dried, and baked at 80°C for 30 min. Prehybridization was performed for 2 h at 42°C in 1× SSC, 5× blocking buffer, 10% SDS, and 10% denatured herring sperm DNA probe, was added, and hybridization was allowed overnight at 42°C. The membrane was rinsed in 2× SSC and washed for 30 min in 0.1× SSC + 0.1% SDS at 65°C. Autoradiography was performed for 2–4 h.

RNA protection analysis. Messenger RNA levels for the EP2 receptor and for rat β-actin were assayed by RNase protection assay basically as described (11). In brief, after linearization with HindIII, the plasmids yielded radiolabeled antisense RNA transcripts by incubation with SP6 polymerase (Promega) and [α-32P]GTP (Amersham Pharmacia Biotech) according to the Promega riboprobe in vitro transcription protocol. RNA probes (5 × 106 counts/min) were hybridized with samples of total RNA at 60°C overnight in a final volume of 50 μl. Sequential digestions were performed with a mixture of RNase A/T1 (Roche, Hvidovre, Denmark) and proteinase K (Roche). The hybrids were separated on 8% polyacrylamide gels. Autoradiography was performed at −80°C for 1–3 days. Radioactivity in the protected probes was quantitated by excision from the gel and β-counting.

Microdissection of rat nephron segments and microvessels. Nephron segments for RT-PCR analysis and for receptor studies were obtained by microdissection of rat kidney tissue from male Sprague-Dawley rats (180–220 g) (36). The rats were anesthetized with mebumal, and both kidneys were perfused through the aorta with 20 ml cold saline followed by 20 ml of DEMEM with 0.1% BSA and collagenase A (1 mg/ml; 0.2 U/mg, Roche). Thin coronal slices were cut, and the slices were incubated in 25 ml DEMEM with 0.1% BSA and 1 mg/ml collagenase A for 25 min at 37°C with modest shaking. Next, the tissue was washed twice with DEMEM containing 10% FCS and kept on ice. Microdissection was done under a stereomicroscope by using sharpened forceps. The nephron segments were identified by their localization in kidney regions and by their appearance. We isolated segments of the proximal convoluted tubule (PCT), proximal straight tubule (PST), descending thin limb of Henle’s loop (DLT) from outer medulla, thin limbs of Henle’s loop (TL) from inner medulla, medullary thick ascending limb of Henle’s loop (mTAL), cortical thick ascending limb of Henle’s loop (cTAL), DCT, CCD, outer medullary collecting duct (OMCD), and inner medullary collecting duct (IMCD). Single glomeruli and preglomerular vessels were isolated by grasping the cortical radial arteries with attached afferent arterioles and carefully removing all tubular tissue and all glomeruli before RNA extraction. For RNA isolation, 30–50 “branching points” were pooled. Vasa recta bundles were dissected from the
outer medulla and were identified on the basis of the typical parallel arrangement of small-diameter structures with irregular cell spacing (see Fig. 7A). Most rat vasa recta bundles are of the simple type that do not contain tubules in the core, but DTL (type 1 cells) can be associated with the bundle periphery in the complex type of bundles (14). We used the core for RNA isolation and carefully inspected the samples to rule out attached tubules. The isolated segments were transferred in 3–5 μl DMEM to 500 μl fresh DMEM in a 24-well cell culture plate on ice. Identical segments were pooled, and the total length of tubule was assessed by a calibrated micrometer scale built into the ocular. For isolation of RNA, at least 10 mm of tubule segments were pooled, except for the DCT segments, of which 5 mm were pooled, and then transferred to 400 μl guanidinium thiocyanate (4 mol/l) solution, and 12 μg yeast tRNA were added as carrier. Samples were stored at −80°C until RNA extraction by the acid-guanidinium-phenol-chloroform extraction protocol (5). RNA from at least 5 mm of microdissected samples was used for RT.

**Incubation studies.** All procedures of anesthesia and surgery were identical to those used above for RNA isolation except for the addition of the cyclooxygenase inhibitor indomethacin (10 μmol/l) to all solutions to inhibit endogenous prostaglandin synthesis. For the incubation studies, the microdissected samples were transferred to 500 μl DMEM containing 0.1% BSA in a cell culture dish on ice. The tubules and vasa recta samples were selected from this pool, and a total length of 2–3 mm (2–5 single tubules) and up to 2 cm of vasa recta (1–2 bundles) were used for incubation, respectively. The samples were stored on ice in a total volume of 20 μl in a 1.5-ml cup until all segments had been distributed. Each experiment was carried out by using segments dissected from one animal. Experiments were only performed when sufficient amounts of tubule segments for a complete dose-response series were obtained no more than 2 h after the animal was killed. The segments were preincubated for 5 min at 37°C in a heating block. Then, 20 μl preheated DMEM mix containing 1 mmol/l of the phosphodiesterase inhibitor IBMX and appropriate test substances were added. The reaction was continued for 5 min. The incubation was terminated by quickly placing the tubes on ice and adding 1 ml of absolute ethanol containing 20 mmol/l HCl. The samples were extracted overnight at −20°C.

Butaprost was a kind gift from Dr. P. L. Gardiner; Bayer, Stoke Court, UK. Butaprost was dissolved in ethanol at a stock concentration of 10−2 mol/l and stored in 20-μl aliquots at −20°C. Ethanol had no effect on cAMP formation in the highest concentrations applied (n = 2). Butaprost was tested in the range of 10−9–10−4 mol/l. The adenylyl cyclase activator forskolin (10−5 mol/l; Sigma, Rødovre, Denmark) was used as a positive control in all experiments. After evaporation of HCl-ethanol in a vacuum centrifuge, cAMP was measured by a commercial RIA kit using the acetylation protocol as suggested by the manufacturer (RPA 509, Amersham-Biotrak, Harsholm, Denmark) as previously described (11).

**Plasma renin concentration.** Renin was measured by ultrimicroassay (16). In short, 10 μl plasma were diluted 20, 40, and 80 times by Tris buffer containing human albumin. Five-microliter samples were incubated for 24 h at 37°C with 20 μl of a mixture of renin substrate, ANG I antibody, and buffer followed by RIA of generated ANG I. Renin concentration is expressed in Goldblatt units (GU) compared with renin standards from the Institute for Medical Research (MRC, Holly Hill, London, UK).

**Statistics.** When several sets of data were compared at the same time (e.g., data from the salt-intake groups), a one-way ANOVA was used. If the ANOVA was significant at the 5% level, differences among data sets were established by using 95% confidence intervals. When two sets of data were compared, an unpaired Student’s t-test was used. P ≤ 0.05 was considered significant.

**RESULTS**

Linearity of the EP2 mRNA assay was tested with total RNA from rat spleen in the 3- to 24-μg range. EP2 receptor expression is reportedly high in spleen (2, 19). Hybridization of the labeled EP2 antisense RNA probe with total RNA from rat spleen resulted in a significant hybridization product, with a size of the hybrid that corresponded to the expected value. Notably, the probe was completely digested in the absence of template RNA, and there was no unspecific hybridization (Fig. 1A, tRNA). The assay was linear in a range from 12 to 24 μg total RNA (Fig. 1A). Significant EP2 expression was detected with the probe in total RNA samples from the inner and outer medulla from rat kidney, whereas expression was not obvious in cortex, with the use of up to 48 μg total RNA for the assay (n = 4, Fig. 1B). β-Actin was used as an RNA loading control, and it was strongly and equally expressed in all kidney regions (Fig. 1B).

Next, EP2 and EP4 receptor expression along the rat nephron was determined by RT-PCR analysis of microdissected rat nephron segments and glomeruli (Fig. 2). Both EP2 and EP4 receptors were expressed in glomeruli; on the basis of serial dilution, EP4 would seem to be much more highly expressed than EP2 (Fig. 2A). In a first screening analysis of nephron segments, EP2...
transcripts were found essentially in the descending medullary part of the loop of Henle: in DTL and in TL dissected from the inner medulla (Fig. 2B, top). The significant amplification product seen in the mTAL segment was falsely positive, as shown later (Fig. 3B). The EP4 receptor was predominantly expressed in DCT and CCD (Fig. 2B, middle). Weaker signals for EP4 were seen in OMCD. Actin was equally amplified in all segments investigated (Fig. 2B, bottom). To confirm the finding on EP2 mRNA localization, separate samples of DTL and mTAL segments obtained in four different animals were analyzed (Fig. 3). EP2 expression was detected in all DTL segments where RT was included, and no amplification products were seen in the absence of RT (Fig. 3A, DTL4-RT) or cDNA. In contrast, the finding of EP2 expression in mTAL could not be reproduced in repeated experiments (Fig. 3B), whereas actin was present in all mTAL samples (Fig. 3B). This discrepancy was most likely caused by contamination of the first mTAL sample with DTL segments.

The correct identification of the EP2- and EP4-positive nephron segments was validated by analysis of marker transcript localization (Fig. 4). AQP1 expression was detected in DTL and TL but not in mTAL (Fig. 4A). The urea transporter UT-A2 was expressed in TL as reported (34) and, unexpectedly, also in mTAL specimens. UT-A2 was not detectable in DTL samples. This identifies our samples as DTL segments from long loops of Henle because UT-A2 is expressed exclusively in short loops of Henle in the outer medulla (34). Actin was expressed equally in all the tested segments (Fig. 4A). The EP4 receptor was found consistently in DCT and CCD, weakly in OMCD, and not found in IMCD (Fig. 4B). The PTH receptor was expressed in DCT, whereas CCD was PTH receptor negative (Fig. 4B), as reported (36). The collecting duct system strongly expressed AQP2 as expected (not shown). Actin was expressed in all the tested segments (Fig. 4B). Thus the EP4 receptor is expressed in the cortical distal nephron and CCD.

To determine whether cAMP-coupled EP2 and EP4 receptors are expressed in the renal resistance vasculature, we dissected out preglomerular vessels from rat
kidney cortex and vasa recta bundles from the outer medulla. RNA was analyzed by RT-PCR and subsequent Southern blotting (Fig. 5). EP4 receptor was strongly expressed in the preglomerular resistance vessels and in outer medullary vasa recta bundles (3 of 3 and 3 of 3 separate preparations, respectively), whereas EP2 receptors were most consistently detected in outer medullary vasa recta bundles (3 of 3 preparations, respectively). In preglomerular vascular samples, one of three PCR reactions was clearly positive for EP2. Rat β-actin was equally amplified from the three preparations of preglomerular vessels and the vasa recta bundle preparations (Fig. 5).

To investigate whether the mRNA localization data were relevant at the level of functional EP2 receptors, we examined the effect of the specific EP2 receptor agonist butaprost on cAMP levels in microdissected nephron segments and outer medullary vasa recta bundles. First, EP2 mRNA-positive DTL segments were compared with EP2 mRNA-negative OMCD segments (Fig. 6). Butaprost significantly increased cAMP formation in DTL (Fig. 6A). A maximal 10-fold stimulation was observed at a concentration of 10⁻⁶ mol/l (control 20.6 ± 8.1 vs. 198 ± 17.1 fmol/mm × 5 min, n = 7). In identical dose-response experiments with OMCD segments, butaprost did not significantly change cAMP production (n = 6; Fig. 6B), and cAMP was at the detection limit of the assay. In contrast, the adenylyl cyclase activator forskolin (10 μmol/l) significantly increased cAMP production in both segments (control 20.6 ± 8.1 vs. 83.4 ± 11.1 in DTL and 6.4 ± 2.2 vs. 135.9 ± 45.4 fmol/mm × 5 min in OMCD, n = 6; Fig. 6). These data suggest that both DTL and OMCD segments are endowed with adenylyl cyclase, but functional EP2 receptors are not present in OMCD.

Subsequently, it was examined whether butaprost affects cAMP levels in outer medullary vasa recta bundles (Fig. 7C). Outer medullary vasa recta bundles and single outer medullary vasa recta (Fig. 7A) expressed the EP2 receptor, and PCR amplification was only noted in the presence of RT (Fig. 7B). Because of the limited amount of tissue that could be dissected in a reasonable time, only a single concentration of butaprost was tested. In individual experiments (n = 3 rats; 2–4 separate samples/condition), butaprost (10⁻⁴ mol/l) invariably increased cAMP production but to different absolute cAMP levels (Fig. 7C). The geometric mean of the control cAMP level was 1.4 vs. 37.8 after incubation with butaprost. Forskolin stimulated basal cAMP levels from 1.4 to 60.2 (geometric mean; data not shown).

In the next series of experiments, we tested the hypothesis that dietary salt intake regulates expression of the EP2 receptor (Fig. 8). After 10 days on high- or low-salt intake, the two groups of rats had plasma renin concentrations that were significantly different (low salt: 2.92 ± 0.5 mGU/ml vs. high salt: 0.14 mGU ± 0.04 mGU/ml; n = 6). Although there was a tendency for a high-salt diet to lower EP2 mRNA abundance in both outer and inner medulla (Fig. 8), this was not statistically significant at the 5% level (P = 0.06). Thus dietary salt content had no effect on the renal medullary EP2 mRNA level.

**DISCUSSION**

We report here on the localization of the cAMP-coupled PGE₂ EP2 and EP4 receptors in rat kidney. EP2 receptor mRNA transcripts were found predominantly in the outer and inner medulla of rat kidney in accordance with our previous report (11). RT-PCR analysis and cAMP measurements showed that the DTL is a major site of EP2 expression along the rat nephron. In contrast, EP4 receptor expression was noted in the outer medullary vasa recta, whereas the EP2 receptor was found most reproducibly in outer medullary vessels.
vasa recta. Glomeruli expressed both EP receptor iso-
forms.

The various effects of PGE2 on kidney function are 
mediated by at least four distinct receptors with differ-
ent intracellular couplings (2, 6, 12, 18, 19, 23, 28, 35).
Recent data suggest that EP2 receptors, but probably 
also EP4 receptors, are involved in PGE2-mediated 
blood pressure regulation, potentially by an effect on 
renal salt handling (13, 30) or vascular reactivity (1, 
39). Previous attempts to localize the EP2 receptor by 
Northern blotting or in situ hybridization of kidney 
tissue from different species have not yielded conclu-
sive evidence (2, 4, 12). More sensitive RT-PCR analy-
sis and immunolocalization showed EP2 expression in 
both rat and human kidney (11, 17, 19). In human 
kidney, the EP2 receptor was not detected in the tubular 
system but only in the smooth muscle of small-caliber 
vessels (17). This finding, which is different from the 
present observations, could reflect species differences 
between rat and human kidneys. Alternatively, it 
might reflect different sensitivities of the methods em-
ployed to detect EP2. The present set of data shows 
agreement between the EP2 localization obtained by 
RNAse protection analysis and by RT-PCR analysis, 
because vasa recta bundles and single vasa recta and 
DTL are restricted to the kidney medulla. Moreover, 
butaprost, a highly specific EP2 receptor agonist with 
low potency (6), markedly and selectively stimulated 
cAMP formation in microdissected DTL segments. In 
agreement, previous reports have shown that PGE2 
potently increases cAMP formation in freshly isolated 
DTL segments from rat kidney (31–33). Thus it is 
justified to conclude that functional EP2 receptors are 
expressed by the DTL in rat kidney. Most of the DTL 
segments that we dissected from the outer medulla 
continued into the inner medulla. The absence of the 
urea transporter UT-A2 in the specimens confirms that 
they belong to long loops of Henle. In the outer me-
dulla, UT-A2 is expressed only in short loops (34). An 
important question regards the functional role the EP2 
receptor plays in the DTL. Medullary PGE2 synthesis

Fig. 5. Localization of EP2 and EP4 re-
ceptor expression in kidney microves-
se. Shown are PCR-amplification 
products for EP2 receptor (top), EP4 
receptor (middle), and for actin (bottom) 
from preglomerular microvessels (PGV) 
and from outer medullary vasa recta 
bundles (OMVB). The figure shows 
ethidium bromide-stained agarose gels 
and corresponding Southern blots. PCR 
was performed for 32 cycles on cDNA 
from 3 separate preparations corre-
spending to 3–5 “branching points” of 
preglomerular vessels or to 1 mm of 
outer medullary vasa recta bundles. As 
a positive control for the RT-PCR, 50 ng 
total RNA from rat spleen for EP2 and 
from rat kidney for EP4 were used. 
H2O, negative control with addition of 
water instead of cDNA. Size marker: 
X174DNA/HaeIII.

Fig. 6. Effect of butaprost on production of cAMP in isolated nephron 
segments. Dose-response relationship between butaprost and cAMP 
levels in DTL (A) and OMCD (B). Segments (2–3 mm/reaction tube) 
of DTL or OMCD for a full dose-response experiment were microdis-
sected from a single rat kidney. The specimens were incubated with 
varying concentrations of butaprost for 5 min. Forskolin (10 μmol/l) 
was used as a positive control of viability in all experiments. Each 
point is the mean ± SE of 6 independent experiments. *P ≤ 0.05.
is increased in many physiological settings, including salt loading (37). To our knowledge, prostaglandin-dependent, cAMP-mediated effects on transepithelial transport of NaCl, water, or urea in the DTL have not been reported thus far. Our data suggest that EP4 receptors are expressed in the distal nephron and early collecting duct system, in DCT and CCD. Previous reports support these data by showing that, in the absence of AVP, PGE2 stimulates cAMP production in cultured DCT cells (7), CCD cells (22, 26), and microdissected CCD segments (31), an effect not mimicked by EP2-specific butaprost (22). Thus altogether the available data suggest the presence of EP4 receptors in DCT and CCD segments. Some data imply segregation of distinct PGE2-coupling mechanisms to different poles of the epithelial cells in this nephron segment. On the one hand, perfusion of CCD segments with PGE2 induces an increase in water permeability (22). On the other hand, abluminal addition of PGE2 inhibits the AVP-induced increase in water permeability (10). The physiological significance of raising cAMP in DCT is related to an enhanced reabsorption of magnesium (7) and probably also calcium.

It is generally believed that PGE2 protects overall renal perfusion. In particular, renal medullary perfusion is markedly sensitive to cyclooxygenase inhibitors under physiological conditions (15, 25). This effect is most likely directly mediated because PGE2 potently dilates isolated and in situ preconstricted afferent arterioles (8, 21, 29), as well as isolated descending vasa recta in vitro (20). Glomerular expression of EP4 receptors has been extensively documented (4, 11, 12, 27), and recently EP2 expression and butaprost sensitivity were also reported in rat glomeruli (9). Our data confirm these observations and extend them to include the major resistance vessel segments. Thus we found EP4 receptor expression in both preglomerular resistance vessels and the vasa recta of the kidney outer medulla, which is in agreement with recent data from rat and human kidney (17, 21, 29). In contrast to EP4, we most consistently detected EP2 receptors in the vasa recta from the outer medulla that, in addition, were sensitive to butaprost. In previous reports (21, 29), EP2 receptors were not found in preglomerular rat vessels. Our data suggest the coexistence of EP2 and EP4 receptors in outer medullary vasa recta. The rea-

In kidney cortex, the EP4 receptor is strongly expressed in glomeruli of several species (4, 11, 12, 27), but tubular sites of EP4 expression have not been identified before. Our data suggest that EP4 receptors are expressed in the distal nephron and early collecting duct system, in DCT and CCD. Previous reports support these data by showing that, in the absence of AVP, PGE2 stimulates cAMP production in cultured DCT cells (7), CCD cells (22, 26), and microdissected CCD segments (31), an effect not mimicked by EP2-specific butaprost (22). Thus altogether the available data suggest the presence of EP4 receptors in DCT and CCD segments. Some data imply segregation of distinct PGE2-coupling mechanisms to different poles of the epithelial cells in this nephron segment. On the one hand, perfusion of CCD segments with PGE2 induces an increase in water permeability (22). On the other hand, abluminal addition of PGE2 inhibits the AVP-induced increase in water permeability (10). The physiological significance of raising cAMP in DCT is related to an enhanced reabsorption of magnesium (7) and probably also calcium.

It is generally believed that PGE2 protects overall renal perfusion. In particular, renal medullary perfusion is markedly sensitive to cyclooxygenase inhibitors under physiological conditions (15, 25). This effect is most likely directly mediated because PGE2 potently dilates isolated and in situ preconstricted afferent arterioles (8, 21, 29), as well as isolated descending vasa recta in vitro (20). Glomerular expression of EP4 receptors has been extensively documented (4, 11, 12, 27), and recently EP2 expression and butaprost sensitivity were also reported in rat glomeruli (9). Our data confirm these observations and extend them to include the major resistance vessel segments. Thus we found EP4 receptor expression in both preglomerular resistance vessels and the vasa recta of the kidney outer medulla, which is in agreement with recent data from rat and human kidney (17, 21, 29). In contrast to EP4, we most consistently detected EP2 receptors in the vasa recta from the outer medulla that, in addition, were sensitive to butaprost. In previous reports (21, 29), EP2 receptors were not found in preglomerular rat vessels. Our data suggest the coexistence of EP2 and EP4 receptors in outer medullary vasa recta. The rea-

is increased in many physiological settings, including salt loading (37). To our knowledge, prostaglandin-dependent, cAMP-mediated effects on transepithelial transport of NaCl, water, or urea in the DTL have not been reported thus far. In the TAL, PGE2 inhibits reabsorption of NaCl through interaction with the EP3 receptor (3), and data suggest that the EP3 receptor itself is regulated by salt intake (11). Although the present data were not statistically significant, there was a tendency for EP2 expression to be decreased by a high-salt intake, and vice versa. A goal of future studies should be an examination of the impact of PGE2 on hydraulic conductivity and on epithelial transport of urea in isolated DTL segments.
son for the existence of two separate cAMP-coupled receptors in this vascular segment is not resolved by the present experiments. The receptors could be located in different cells, because the descending vasa recta consist of both a continuous layer of endothelial cells that control water, NaCl, and urea transport and a layer of contractile pericytes involved in regulation of medullary vascular resistance. Thus it is possible that EP2 receptors are involved in PGE2-mediated regulation of water and urea permeability of DTL and vasa recta, whereas EP4 receptors are involved in PGE2-mediated regulation of vascular tone.

This work was supported by grants from the Danish Health Science Research Council (9903058, 9902742), the Novo Nordisk Foundation, the Foundation of 23–9–1909, the Danish Heart Association (98–1–2–8–25283, 99–2–2–36–22743), the Ms. Ruth T. E. König Petersens Research Foundation for Kidney Diseases, the Danish Medical Association Research Fund, and the Overlægerådets Legatudvalgs Fond.

REFERENCES


