Low-dose indomethacin after ischemic acute kidney injury prevents downregulation of Oat1/3 and improves renal outcome

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Submitted 15 May 2009; accepted in final form 8 September 2009

The organic anion transport system of the renal proximal tubule plays a crucial role in the excretion of a variety of potentially toxic compounds (25, 37). This system consists of organic anion exchangers located at the basolateral membrane representing the rate-determining step of elimination and the efflux step through the apical membrane (7, 10). The classical basolateral organic anion exchanger is the terminal step in a tertiary active transport system, dependent on an inward-directed Na+ gradient to drive the uptake of α-ketoglutarate, which is then exchanged for organic anions (13, 41). It has been shown that organic anion transporter (Oat) 1 and Oat3 represent characteristics of the basolateral, polyspecific transporter for organic anions (21, 33), which had been functionally described for a substantial amount of time (41). In summary, the classical renal basolateral polyspecific uptake transporter for organic anions is represented by Oat1 and Oat3 (8, 32).

Meanwhile, there is increasing evidence that renal basolateral organic anion uptake is regulated. An increasing number of studies published concentrate on acute regulatory phenomena and deal with regulation by protein kinase C and protein kinase A (for review, see Refs. 8 and 34). With respect to long-term regulation of renal organic anion transport, much less data are available. There is evidence that renal organic anion transport is regulated by sex steroids (27), which is now shown to be most probably due to regulation of Oat1 and Oat3 (4, 22). Moreover, hyperuricemia was shown to reversibly downregulate Oat1 and Oat3 (14, 15). In the rat kidney, Oat1 and Oat3 are both downregulated after ureteral obstruction (39).

In human renal allograft, the clearance of the prototypical organic anion para-aminohippurate (PAH) was reduced for at least 7 days after transplantation (5). Based on the latter observation, we performed a study showing downregulation of both Oat1 and Oat3 during reperfusion after ischemic acute kidney injury (iAKI) (31). This was subsequently confirmed by independent groups (9, 23).

We have additionally shown that prostaglandin E2 (PGE2) leads to downregulation of the expression of both Oat1 and Oat3 in the rat proximal tubular cell line NRK-52E after long-term exposure (up to 72 h) (29). It has been shown that PGE2 levels are increased in kidney cortex after acute renal ischemia (24, 36) or during chronic renal ischemia (35). There has moreover been evidence that impairment of PGE2 formation has beneficial effects on renal outcome after iAKI (11, 12).

Based on all this, we hypothesized that PGE2 generated in renal cortex during iAKI leads to impaired expression of Oat1 and Oat3. If this would be the case, inhibition of ischemia-reperfusion (I/R)-induced generation of cortical PGE2 should be able to avoid or at least reduce downregulation of Oat1 and Oat3. Thus we investigated the effect of the nonselective cyclooxygenase (COX) inhibitor indomethacin applied after renal ischemia on the expression of the latter transporters, on renal transport of the organic anions PAH and PGE2, and on basal parameters of kidney function [glomerular filtration rate (GFR) and renal plasma flow (RPF)].
In vivo experimental procedure. Experiments were performed as published recently (26, 30), where I/R injury was induced by bilateral clamping of renal arteries for 45 min in rats. Female Sprague-Dawley rats (200–250 g body wt) were obtained from Charles River (Kisseleg, Germany). After a period of at least 24 h in cages within a temperature-controlled room with a 12:12-h light-dark cycle and standard food with free access to tap water, anesthesia was performed by intraperitoneal application of xylacine hydrochloride (10 mg/kg body wt) and ketamine (100 mg/kg body wt). All operative procedures were performed on thermoregulated heating boards to maintain body temperature at 37.0°C. Postoperative pain relief was assured by subcutaneously applied tramadol (0.05 mg/kg body wt), and postoperative dehydration was prevented by subcutaneous administration of additional 1.0 ml 0.9% NaCl. Animals were divided into the following subgroups.

Clamping group (bilateral clamping and supplementation with saline). Both kidneys were prepared carefully by bilateral flank incision. Renal arteries were dissected and temporarily ligated on both sides to start clamping with microclips simultaneously.

Sham group (sham operation and supplementation with saline). An identical procedure was performed in analogy as described for the clamping group, except that no clamping of renal arteries was performed.

Clamping group (resp. sham group) receiving indomethacin. Indomethacin was given at 1 mg/kg ip 10 min before the end of the clamping (or after sham operation) period to assure immediate delivery in the kidney right at the beginning of reperfusion and to exclude possible renal effects of indomethacin already during ischemia.

Control group (untreated animals). Animals with no previous treatment were investigated. These animals reflect day 0.

The care of animals and experimental procedures performed in this study were in accordance with the German law for animal protection.

Measurement of clearances of inulin (GFR) and PAH (RPF). Inulin concentrations of urine and plasma samples were determined collected for 20 min, and blood samples were drawn subsequently. PAH) using a Secura FT perfusor (B. Braun, Melsungen, Germany).

mg/ml 0.9% NaCl) were initially applied as a bolus injection, fol-

brief, fluorescein-isothiocyanate-inulin (inulin) and PAH (each 2.5

and PAH clearances were determined as described recently (13). In this study were in accordance with the German law for animal protection. Conditions (100 mmHg) with ice-cold Krebs buffer [in mM: 118 NaCl, 25 NaHCO3, 4.8 KCl, 1.2 KH2PO4, 1.2 MgSO4, 11 glucose, and 1.5 CaCl2(2H2O)] for 20 s. Subsequently, samples of renal cortex were snap-frozen in liquid nitrogen and stored at −80°C.

Protein immunoblot. For Western blot analysis, frozen kidney cortex was homogenized using a stainless steel mortar cooled by liquid nitrogen, dissolved in lysis buffer containing 25 mmol/l Tris·HCl, 7 mmol/l reduced glutathione, 0.5 mmol/l EDTA, 0.2 mol/l phenylmethylsulfonyl fluoride, 1 μmol/l leupeptin, 1 μmol/l pepsta-

tin, 1 μmol/l trans-epoxysuccinyl-l-leucylamido butane, and 1 mg/ml trypsin inhibitor and further minced with an ultrasonic disperser UW 70 (Bandelin Electronic, Berlin, Germany). Total protein was mea-

sured in samples using the Bradford (3) method. Samples of protein (5–40 μg) were analyzed by Western blot with the respective antibodies. Rabbit anti-rat Oat1 polyclonal antibody (diluted 1:500) and rabbit anti-rat Oat3 polyclonal antibody (diluted 1:500) were from alpha diagnostic (San Antonio, TX). Preincubation of the antibodies to the respective blocking peptides led to disappearance of the bands, as already presented and discussed previously (31). Blots were subse-

sequently incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:2,000; Dako, Hamburg, Germany) and were developed using an ECL chemiluminescence kit (ECL Plus) following the manufacturer’s instruction (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Blots were analyzed densitometrically using the Quantity One software (Bio-Rad Laboratories, Philadelphia, PA).

Real-time RT-PCR. RNA from kidney cortex was extracted using the Qiagen RNA Isolation Kit (Qiagen, Hilden, Germany). RNA concentration was determined, and cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer’s instructions. In brief, RT-PCR was performed according to the iQ SYBR-Green Supermix RT-PCR system protocol (Bio-Rad). Initial denaturation was performed at 95°C for 3 min. PCR amplification was performed in 45 cycles of 94°C for 15 s, then 55°C for 30 s, and 72°C for 60 s. The final elongation step was 72°C for 10 min. For Oat1, the primers were 5′-aga gtc atc gag ccc tgc at-3′ (sense) and 5′-gcc cag gct gta gag ata gc-3′ (antisense), resulting in an 402-bp RT-PCR product. For Oat3, the primers were 5′-tct tgg tgg gta cca gc-3′ (sense) and 5′-ctg cat ttc tca agg cca aa-3′ (antisense), resulting in a 468-bp RT-PCR product. For β-actin, the primers were 5′-tct tgg atc agc gag ccc tgc atg-3′ (sense) and 5′-tac gtc gtt ggg tgg gaggc aa-3′ (antisense), resulting in an 102-bp RT-PCR product. The RT-PCR products generated with primers for Oat1 and Oat3 were sequenced by MWG Biotech, München, Germany) and were found to represent the predicted parts of the respective mRNAs. The RT-PCR products were tested for correct size by agarose gel electrophoresis and melting point analysis. Quantification was performed using the ΔΔCT method using β-actin as a reference gene, and expression in sham animals was normalized to one.

PGE2 transport. PGE2 in the supernatant was determined by competitive enzyme-linked immunosorbent assay technique using the Correlate-EIA PGE2 Enzyme Immunoassay Kit from Assay Designs (Ann Arbor, MI). Measurement and calculation was done in a 96-well plate according to the manufacturer’s instructions. In brief, 100 μl of either sample were given in a single well, and 100 μl of a supplied solution containing conjugate (PGE2 attached covalently to alkaline phosphatase) and PGE2 antibody were added. The plate was incubated at room temperature for 2 h on a shaker, and wells were washed three times afterwards. Next, 200 μl substrate solution were added for 45 min, and subsequently 50 μl stop solution were added before optical density was determined at 405 nm in a multiwell-multilabel counter (Victor2; Wallac, Turku, Finland). PGE2 in the supernatant was then calculated and referred to the amount of cell protein per well. PGE2 clearance was calculated in accordance to what is described above for PAH. PGE2 secretion is calculated as the amount of PGE2 in the urine in relation to the amount of inulin therein.

Detection of invading monocytcs in renal cortex. Immunofluorescence detection of invading ED-I positive cells was done similar as previously described in detail (26). In brief, cryostat sections (5 μm) were fixed in PBS buffer with 4% paraformaldehyde at a temperature of 4°C. Sections were permeabilized with 0.1% Triton X-100, and antibodies were incubated overnight at 4°C. After washing, sections were incubated with a fluorochromelabeled secondary antibody and mounted with a fluoresecin isothiocyanate (FITC)-or rhodamine isothiocyanate (RITC)- conjugated secondary antibody. Sections were examined with a Zeiss Axioscope microscope equipped with epifluorescence illumination.

Expression of Oat1 and Oat3. The expression of Oat1 and Oat3 was analyzed by real-time quantitative RT-PCR. Total RNA was isolated from renal cortex using the Qiagen RNA Isolation Kit (Qiagen, Hilden, Germany). RNA concentration was determined, and cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer’s instructions. In brief, RT-PCR was performed according to the iQ SYBR-Green Supermix RT-PCR system protocol (Bio-Rad). Initial denaturation was performed at 95°C for 3 min. PCR amplification was performed in 45 cycles of 94°C for 15 s, then 55°C for 30 s, and 72°C for 60 s. The final elongation step was 72°C for 10 min. For Oat1, the primers were 5′-aga gtc atc gag ccc tgc at-3′ (sense) and 5′-gcc cag gct gta gag ata gc-3′ (antisense), resulting in a 402-bp RT-PCR product. For Oat3, the primers were 5′-tct tgg tgg gta cca gc-3′ (sense) and 5′-ctg cat ttc tca agg cca aa-3′ (antisense), resulting in a 468-bp RT-PCR product. For β-actin, the primers were 5′-tct tgg atc agc gag ccc tgc atg-3′ (sense) and 5′-tac gtc gtt ggg tgg gaggc aa-3′ (antisense), resulting in an 102-bp RT-PCR product. The RT-PCR products generated with primers for Oat1 and Oat3 were sequenced by MWG Biotech, München, Germany) and were found to represent the predicted parts of the respective mRNAs. The RT-PCR products were tested for correct size by agarose gel electrophoresis and melting point analysis. Quantification was performed using the ΔΔCT method using β-actin as a reference gene, and expression in sham animals was normalized to one.

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of 4°C for 10 min. After being rinsed with PBS, buffer sections were blocked with 50 mM NH₄Cl for another 10 min, followed by another rinsing in PBS. Additionally, sections were incubated with 0.1% Triton X-100 in PBS buffer for 10 min. Finally, they were blocked with 10% donkey serum in 0.1% Triton X-100 in PBS buffer for 1 h. Subsequently, the anti-rat macrophage antibody ED1 (CD68 antibody; Acris BM 4000, Herford, Germany) was incubated 1:400 in 10% donkey serum in PBS buffer, followed by donkey anti-mouse Cy3-conjugated secondary antibody (1:500; model 715–165-151; Dianova, Hamburg, Germany) in 10% donkey serum for 1 h. After a last rinse in PBS and H₂O, analysis of renal cortex was performed using an epifluorescence microscope (NIKON Eclipse TE 2000-S). Final analysis was performed by manually counting the number of ED-1 positive cells in one randomly defined visual field of renal cortex.

Data analysis. Data are presented as means ± SE. The n value is given in the text or in Figs. 1–7. For all experiments, n equals the number of rats or the number of experiments (RT-PCR, Western blot) with tissue or tissue extractions from distinctive rats. Statistical significance was determined by unpaired Student’s t-test. Data from sham-operated animals were tested against untreated controls, and data from clamped animals were tested against sham-operated animals. Differences were considered statistically significant when P < 0.05.

Materials. Tramadol (Tramal) was from Grünenthal (Aachen, Germany), xylacin hydrochloride (Rompun) was from Bayer (Leverkusen, Germany), and ketamine (Ketanest) was from Pharmacia & Upjohn (Erlangen, Germany). If not indicated otherwise, all substances were further diluted in 0.9% NaCl (wt/vol). If not stated otherwise, chemicals were from Sigma (St. Louis, MO).

RESULTS

Effect of indomethacin on expression of Oat1 and Oat3. As already mentioned in the introduction, we hypothesized that PGE₂ generated after renal ischemia leads to downregulation of both Oat1 and Oat3. Thus inhibition of COXs should avoid generation of PGE₂ and thereby circumvent downregulation of Oat1 and Oat3. Indomethacin alone (1 mg/kg) applied directly after ischemia (see MATERIALS AND METHODS for details) had no effect on the amount of mRNA of either Oat1 or Oat3 after 24 h reperfusion compared with the sham-operated control. Indomethacin more than doubled PAH clearance in clamped animals. Thus inhibition of COXs should avoid generation of PGE₂ and thereby circumvent downregulation of Oat1 and Oat3. Indomethacin alone (1 mg/kg) applied directly after ischemia compared with the sham-operated animals. Thus, in rats, 1 mg/kg indomethacin has a substantial effect on PAH secretion.

Effect of indomethacin on parameters of renal function. Because there is sporadic evidence that indomethacin might have beneficial effects on renal outcome after iAKI (11, 12), we investigated GFR (inulin clearance) and RPF (PAH clearance) 24 h after iAKI. Most interestingly, indomethacin attenuated the decrease of GFR 24 h after renal ischemia in clamped animals (Fig. 4), leading to a more than doubled renal filtration compared with clamped animals not treated with indomethacin. However, GFR is still lower compared with untreated controls (0 h) and in sham-operated animals. Because indomethacin did not lead to increased expression of Oat1 or Oat3 in sham-treated animals, the observed increase in PAH secretion must be due to an effect different from the one on expression of Oat1 and Oat3. Nevertheless, the data on expression of Oat1 and Oat3 correlate well with the functional description of organic anion (PAH) secretory capacity.

Effect of indomethacin on PAH secretion. As presented in Fig. 3, iAKI-induced reduction of PAH net secretion is avoided in part by application of 1 mg/kg indomethacin after ischemia compared with the sham-operated animals also receiving indomethacin. Indomethacin alone led to an increase in PAH net secretion compared with the sham-treated animals. However, in clamped animals, indomethacin increased PAH net secretion to a range that is found in untreated controls (0 h) and in sham-operated animals. Because indomethacin did not lead to increased expression of Oat1 or Oat3 in sham-treated animals, the observed increase in PAH secretion must be due to an effect different from the one on expression of Oat1 and Oat3. Nevertheless, the data on expression of Oat1 and Oat3 correlate well with the functional description of organic anion (PAH) secretory capacity.

Fig. 1. Effect of 1 mg/kg indomethacin on the mRNA levels of organic anion transporter (Oat) 1 and Oat3 in renal cortex from Sprague-Dawley rats 24 h after renal ischemia. Total RNA was generated from kidney cortex. Real-time PCR against Oat1, Oat3, and β-actin was performed as described in MATERIALS AND METHODS. Quantification was performed using the ΔΔCt method using β-actin as a reference gene, and expression in untreated control animals (con 0 h) was normalized to 1. A: effect of 1 mg/kg indomethacin on the mRNA levels of Oat1. The amount of Oat1 mRNA signal normalized to the respective signal from β-actin. *Statistically significant difference between sham-operated and clamped animals. The no. of experiments (n) is given in the respective bars. B: effect of 1 mg/kg indomethacin on the mRNA levels of Oat3. The amount of Oat3 mRNA signal normalized to the respective signal from β-actin. *Statistically significant difference between sham-operated and clamped animals; n is given in the respective bars.
animals. Indomethacin alone had no effect. Because PAH clearance is dependent both on PAH secretion in the proximal tubule and on renal perfusion, this may be due to improved PAH secretion only. However, from our data, it is not possible to decide whether and to what extent renal perfusion is really improved by indomethacin.

### Effect of indomethacin on renal transport of PGE2

It is well known that PGE2 is generated in renal cortex after ischemia (24, 36). Moreover, PGE2 is known to downregulate expression of Oat1 and Oat3 (29) and is additionally known to be an endogenous substrate for organic anion transporters itself (19). Thus secretion of the endogenous organic anion PGE2 should be impaired after I/R injury, and indomethacin should be able to avoid this particular impairment. Actually, we found that PGE2 clearance is diminished after ischemia and 24 h reperfusion (Fig. 6A), and this is not the case if indomethacin was applied directly after renal ischemia. Due to what was shown for PAH net secretion, this should be due to effects on PGE2 clearance.

### Materials and Methods

#### Western Blotting

- **Fig. 2.** Effect of 1 mg/kg indomethacin on the protein levels of Oat1 and Oat3 in renal cortex from Sprague-Dawley rats 24 h after renal ischemia. Total protein was generated from kidney cortex. Western blot against Oat1 and β-actin was performed as described in MATERIALS AND METHODS. Western blotting was performed using protein extracts from kidney cortex either from sham-operated or from clamping treated with 1 mg/kg indomethacin directly after ischemia. Untreated animals served as controls. *Statistically significant difference between sham-operated and clamped animals; n is given in the respective bars. A: effect of 1 mg/kg indomethacin on the relative expression of Oat1. Antibody against Oat1 recognized a band in the range of 57 kDa; the anti-β-actin antibody recognized a band at 42 kDa. The amount of Oat1 Western blot signal was normalized to the respective signal from β-actin. Because β-actin signal was unaffected, it is not shown. B: effect of 1 mg/kg indomethacin on the relative expression of Oat3. Antibody against Oat1 recognized a band in the range of 110 kDa; the anti-β-actin antibody recognized a band at 42 kDa. The amount of Oat1 Western blot signal was normalized to the respective signal from β-actin. Because β-actin signal was unaffected, it is not shown.

#### Renal Net Secretion and Clearance

- **Fig. 3.** Effect of 1 mg/kg indomethacin on the renal net secretion (PNS) of para-aminohippuric acid (PAH) in Sprague-Dawley rats 24 h after renal ischemia. Renal net secretion of PAH (PNS) was determined described in MATERIALS AND METHODS. PNS = [([PAHU × VU]/t) - ([GFR × PAHP])], where PAHU is PAH concentration in urine, PAHP is PAH concentration in plasma, VU is urine volume, t is time of measurement, and GFR is glomerular filtration rate. After either a sham operation or a bilateral clamping of the renal arteries for 45 min, 1 mg/kg indomethacin (ip) was applied or not, and the renal net secretion of PAH was determined 24 h afterwards. Renal net secretion of PAH was additionally determined in untreated control rats; n is given in or beside the respective bars. *Statistically significant difference from sham-operated animals. #Statistically significant difference from control animals. +Statistically significant difference as indicated.

- **Fig. 4.** Effect of 1 mg/kg indomethacin on the renal clearance of inulin in Sprague-Dawley rats 24 h after renal ischemia. Renal clearance of inulin was determined as a measure of GFR as described in MATERIALS AND METHODS. GFR = ([IU × VU]/IP × t), where IU is inulin concentration in urine, and IP is inulin concentration in plasma. After either a sham operation or a bilateral clamping of the renal arteries for 45 min, 1 mg/kg indomethacin (ip) was applied or not, and the clearance of inulin was determined 24 h afterwards. Inulin clearance was additionally determined in untreated control rats; n is given in or beside the respective bars. *Statistically significant difference from sham-operated animals. #Statistically significant difference from control animals. +Statistically significant difference as indicated.
secretion itself. Therefore, we determined the ischemia-induced changes of PGE2 in urine (compared with the respective sham groups) normalized to inulin (pg/μg) as a measure of changes in PGE2 secretion. Because indomethacin was applied systemically, PGE2 in plasma was diminished to ~40% compared with non-indomethacin-treated animals (in ng/ml plasma: sham: 5.6 ± 1.5; clamp: 5.2 ± 1.0; sham + indomethacin: 2.2 ± 0.6; clamp + indomethacin: 2.1 ± 0.8). This is likely the cause for the difference in total PGE2 excretion between sham animals (1,477 ± 321 pg/μg) and sham animals receiving indomethacin (496 ± 114 pg/μg). As presented in Fig. 6B, however, renal PGE2 secretion itself is inhibited ~60% after ischemia, whereas this is not the case if indomethacin was added together with reperfusion. This is evidence that renal ischemia via downregulation of Oat1 and Oat3 also decreases renal secretion of an organic anion from endogenous origin (PGE2) 24 h after ischemia. When downregulation of the latter transporters is abolished by indomethacin, organic anion secretion (PAH or PGE2) is not impaired.

**Effect of indomethacin on invasion of monocytes in renal cortex.** It is well known that monocytes/macrophages invade in renal cortical tissue after ischemia, which is part of an inflammatory response, and detection as ED-1-positive cells is well described (38, 40). Thus the amount of renal cortical ED-1 was determined as an estimate of renal inflammatory response after ischemia and 24 h reperfusion. Therefore, we investigated the effect of indomethacin on monocyte/macrophage invasion. As suspected, ischemia increased the amount of ED-1-positive cells detected in renal cortex. Remarkably, however, 1 mg/kg indomethacin has no effect on ischemia-induced cell invasion (Fig. 7). This indicates that the beneficial effect on renal outcome induced by low-dose indomethacin is likely not due to its anti-inflammatory potency.

**DISCUSSION**

In the present study, we addressed a question that emerged as a consequence of what we have shown before (29, 31): Is impaired expression of Oat1 and Oat3 after I/R injury due to a downregulation induced by PGE2? If this would be the case, impairment of I/R-induced generation of cortical PGE2 should avoid or at least reduce downregulation of Oat1 and Oat3. Therefore, we investigated the effect of the nonselective COX inhibitor indomethacin applied during reperfusion after renal ischemia on the expression of the latter transporters, on renal
transport of organic anions from exogenous and endogenous origin, and on basal parameters of kidney function.

Investigation of the time response of organic anion transporter expression and renal function after renal ischemia (31) showed a stable maximum detrimental effect from 6 h to up to 24 h after renal ischemia. Therefore, we decided to concentrate on a reperfusion interval of 24 h in the present study. The effect of ischemia observed after 24 h reperfusion is in good agreement with data published previously (26, 31), indicating a good reliability and reproducibility of this particular in vivo model system of iAKI. Indomethacin was given intraperitoneally directly after ischemia as a single dose of 1 mg/kg body wt. This particular dosage was chosen to minimize potential impairment of renal perfusion, since renal perfusion mainly in the medulla is described to be dependent on PGE2 (16). Because PAH clearance in sham-treated animals is not affected by the latter amount of indomethacin, there obviously is no substantial effect on renal perfusion. However, because >25-fold accumulation of indomethacin is described in rat proximal tubules (6), this should result in an intracellular proximal tubular concentration of ~70 µM, which is sufficient to inhibit both COX1 (IC50 ~0.3 µM) and COX2 (IC50 ~7.7 µM), even if any other renal concentrating mechanisms than proximal tubular accumulation are neglected.

As indicated by qPCR and Western blot, indomethacin really avoided I/R-induced downregulation of both Oat1 and Oat3. This is in good agreement with the working hypothesis developed in detail in the introduction, indicating that I/R-induced PGE2 leads to downregulation of Oat1 and Oat3. Thus indomethacin should also improve net secretion of the classic organic anion transporter substrate PAH, since both transporters mediate the rate-limiting step of renal organic anion secretion (7, 10). This is indeed clearly indicated by measurements of PNS. Only recently, H"oc"erl and coworkers (17) have shown similar effects on the expression of Oat1 and Oat3 and the organic anion secretion 12 h after renal ischemia using 20 mg/kg of the COX2 inhibitor parecoxib. Although there are some differences to our respective study (COX2 inhibitor at high dose given 1 h before a 30-min ischemia with 12 h reperfusion), the data presented therein are also in accordance with the idea of a PGE2-induced downregulation of Oat1 and Oat3 after renal ischemia. Additionally, the authors describe the ischemia-induced increase of PGE2 in the renal cortical tissue during reperfusion, which is impaired by parecoxib. Together with the fact that PGE2 leads to downregulation of the expression of both Oat1 and Oat3 in the rat proximal tubular cell line NRK-52E (29), this is strong evidence that PGE2 also downregulates expression of Oat1 and Oat3 after renal ischemia in vivo. However, this certainly is no real proof, since other eicosanoids generated by COX activity might possibly do the same. Future studies will have to investigate this in more detail.

PGE2 is an exemplary endogenous substrate for renal organic anion transport (19) that mediates important physiological functions of the kidney and that is moreover of pathophysiological relevance (16). Therefore, we investigated renal handling of PGE2 showing I/R-induced reduction of PGE2 clearance, which is avoided by indomethacin. Even more impressive, I/R led to a decrease in renal PGE2 secretion, and this decrease is also abolished by COX inhibition. Thus, although PGE2 is increased in renal cortex during reperfusion after ischemia (17, 24, 36), the amount of PGE2 secreted in the tubular lumen is depleted. Thus, in accordance to what was shown for secretion of PAH, the secretory transport of another organic anion from endogenous origin (PGE2) is impaired during reperfusion after ischemia, and this is abolished by indomethacin.

Because waste removal impairment is thought to contribute to renal I/R damage (28), downregulation of Oat1/3 via accumulation of metabolic (anionic) end products may play a role therein. Thus maintenance of organic anion secretion after ischemia by indomethacin potentially will reduce I/R damage. If this speculation will hold true will be tested in future studies. However, if it actually does, it may represent a new mechanistic concept for the explanation of renal damage during reperfusion after acute ischemia. Moreover, inflammation is thought to play an important role in I/R damage (2, 20). Thus, in principle, indomethacin also might reduce I/R damage because of its anti-inflammatory potency and thus improve renal outcome after iAKI. However, low-dose indomethacin did not prevent ischemia-induced monocyte invasion, as shown in Fig. 7. Because invasion of ED-1-positive cells is thought to be a part of the inflammatory response after renal ischemia (26, 38, 40), this indicates that the beneficial effect on renal outcome induced by posts ischemic application of 1 mg/kg indomethacin is not due to an inhibition of inflammation.

To describe renal outcome after ischemia, we investigated the clearance of PAH and insulin as parameters describing general renal function (RBF and GFR). As already mentioned, it is hardly possible to decide whether and to what extent renal perfusion is really improved due to indomethacin, since PAH secretion itself is improved. However, indomethacin substantially abolished the decrease of GFR 24 h after renal ischemia, really indicating an amelioration of renal outcome. It was suggested that late (24 h after ischemia) reduction of GFR after iAKI is mediated, at least in part, by tubuloglomerular feedback (TGF) (18). Moreover, it is known that COX activity products partly mediate TGF (1). Although only speculative, this is another possible explanation for the beneficial effects of indomethacin on GFR 24 h after ischemia.

Taken together, PAH clearance and GFR were positively influenced by indomethacin, indicating a protective role of
COX inhibition, which was already implicated by some other the studies (11, 12). However, therein, indomethacin was applied already before induction of ischemia. Thus our data are the first evidence that low-dose indomethacin (1 mg/kg) applied directly after ischemia has a beneficial effect on renal outcome after ischemia. This indicates that, during reperfusion, COX metabolites, namely PGE2, play an important role in the development or maintenance of renal damage and that pharmacological intervention may be a therapeutically relevant option after acute ischemic damage of the kidney or after kidney transplantation. Whether the apparent improvement of function also corresponds to diminished damage of renal tissue will be investigated in future studies. Moreover, we have obtained some evidence that the beneficial effect of low-dose indomethacin is due to an effect different from inhibition of inflammation. In the future, we will investigate if the hypothesis that the beneficial effect of low-dose indomethacin is due to its restoring effect on organic anion transport holds true.

ACKNOWLEDGMENTS

This work is in part supported by the Interdisziplinäres Zentrum für Klinische Forschung Würzburg Grant E-34(1).

DISCLOSURES

No conflicts of interest are declared by the authors.

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AJP-Renal Physiol • VOL 297 • DECEMBER 2009 • www.ajprenal.org


