Exogenous 5'-nucleotidase improves glomerular autoregulation in Thy-1 nephritic rats

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clonal anti-Thy-1 antibody on renal autoregulation. In the present study, we assessed the role of mesangial cells in vivo renal autoregulation, comparing controls and Thy-1 nephritis induced by mAb1–22-3, another monoclonal antibody against Thy-1, which recognizes a Thy-1 epitope different from OX-7, one that is more nephrotoxic than OX-7 (25).

METHODS

Experiments were performed using 49 Wistar-Kyoto (WKY) rats weighing 210–340 g (Shizuoka Laboratory Animal Center, Shizuoka, Japan). The animals were allowed to freely access tap water and standard rat chow (CE-2, Nihon CLEA, Tokyo, Japan). All experimental protocols were approved by our institutional ethical committee. A mouse monoclonal antibody to rat Thy-1 antigen (mAb1–22-3; 2 mg) or control (2 mg murine IgG in 1 ml saline) was intravenously administered to the rats. We used mAb1–22-3 because our previous data demonstrated that it was more effective than OX-7 in inducing mesangioysis (25). Two days later (29), the rats were anesthetized with pentobarbital sodium (50 mg/kg ip) and placed on a thermostatically controlled heated table to keep body temperature at 37°C, as detailed previously (24, 38). A tracheostomy was performed, and the right jugular vein was cannulated with polyethylene tubing (PE-50) to allow infusion of solutions and additional anesthetic. The animals were infused at the rate of 1.2 ml/h with an isotonic saline solution containing 6% BSA (Sigma, St. Louis, MO) during surgery and thereafter with an isotonic saline solution containing 1% BSA, 7.5% Inunest (Laevosan-Gesellschaft, Linz/Donau, Austria), and 1.5% PAH (Merck Sharp & Dohme, West Point, PA). The left femoral artery was cannulated with PE-50 filled with heparinized saline (100 U/ml) to allow blood sampling and continuous arterial pressure measurements with a transducer (DX-100, Nihon Kohden, Tokyo, Japan) and a polygraph recorder (RM-7000, Nihon Kohden). The abdomen was opened by a midline incision. The left ureter was cannulated (PE-10), and urine was collected under mineral oil in preweighed tubes. An adjustable clamp was placed on the aorta above the left renal artery (and below the right renal artery) to control left renal arterial pressure. For 5′-nucleotidase experiments, the left adrenal artery was cannulated with extended PE-10 to infuse heparinized saline or 5′-nucleotidase at a rate of 0.6 ml/h (41), and the solution for transjugular infusion was altered to one containing 2% BSA and infused at the rate of 0.6 ml/h to make the water load similar. Rats were allowed to breathe air enriched with oxygen (100% O2), which markedly improves the stability of arterial blood pressure. After completion of the surgery, a 1-h equilibration period was allowed before experimental protocols were initiated.

In four separate groups of rats, renal clearance studies were performed. The first series of experiments was performed in six control and six nephritic rats (group 1). Initially, two consecutive 30-min control clearances were carried out. Then, renal arterial pressure was reduced by ~20 mmHg. A 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. The aortic clamp was released. The solution infused intravenously was changed to an isotonic saline solution containing 30% BSA, 7.5% Inunest, 1.5% PAH, and furosemide (16 μg·kg⁻¹·min⁻¹) to inhibit TGF without changes in blood pressure (12, 26). Another 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Subsequently, the aortic clamp was tightened to reduce renal arterial pressure by 20 mmHg. Then, two final consecutive 30-min clearance studies were carried out.

In the second study, to test whether 5′-nucleotidase may modify renal autoregulation, the effects of 5′-nucleotidase (Sigma) on renal hemodynamics were examined using six control and six nephritic rats (group 2). Initially, saline was infused into the left renal artery through the adrenal artery. Two consecutive 30-min control clearances were carried out. Then, the aortic clamp was tightened. A 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. The aortic clamp was released. Inert saline was changed to 5′-nucleotidase-containing saline and infused into the renal artery at the rate of 0.03 U/min. Another 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Subsequently, the aortic clamp was tightened to reduce renal arterial pressure by 20 mmHg. Finally, two consecutive 30-min clearance studies were carried out. Preliminary data suggested that 5′-nucleotidase is such a strong renal vasoconstrictor that 5′-nucleotidase decreased renal plasma flow (RPF) even in control rats at doses of 0.1 U/min and above. Although both 0.01 and 0.03 U/min of 5′-nucleotidase infusion failed to alter RPF (3.88 ± 0.37 to 3.83 ± 0.43 and 3.73 ± 0.27 ml·min⁻¹·g kidney wt⁻¹, respectively), RPF declined (to 3.42 ± 0.33 ml·min⁻¹·g kidney wt⁻¹, P < 0.05, n = 4) during 0.1 U/min of 5′-nucleotidase infusion into normal rat kidneys. We applied the highest dose of 5′-nucleotidase that did not affect normal rats (0.03 U/min).

In complementary studies, the effects of 8-cyclopentyl-1,3-dipropylxanthine (CPX), a selective adenosine-1 receptor antagonist, on renal vasoconstriction induced by 5′-nucleotidase were examined in five control rats (34). Initially, saline was infused into the left renal artery through the adrenal artery. Two consecutive 30-min control clearances were carried out. Inert saline was changed to 5′-nucleotidase-containing saline and infused into the renal artery at the rate of 0.3 U/min. A 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Then, CPX (0.1 mg/kg) was intravenously administered. Another 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Subsequently, CPX (0.3 mg/kg) was intravenously administered. Finally, two consecutive 30-min clearance studies were carried out. CPX was purchased from Sigma, and a stock solution (2.5 mg/ml) was freshly prepared with 0.1 M NaOH in saline on the day of each experiment (3).

In the third series of experiments (group 3), the effects of nitric oxide synthesis inhibition on renal hemodynamics in nephritic rats (n = 5) were examined. Initially, two consecutive 30-min control clearances were carried out. Then, renal arterial pressure was reduced by ~20 mmHg. A 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Then, the transjugular solution was changed to an isotonic saline solution containing 1% BSA, 7.5% Inunest, 1.5% PAH, and nitro-l-arginine (0.2 mg·kg⁻¹·min⁻¹, Sigma) to inhibit nitric oxide synthesis (38). The aortic clamp was only partially released to render renal arterial pressure similar to the basal level. Another 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Subsequently, the aortic clamp was tightened to further reduce renal arterial pressure by 20 mmHg. Finally, two consecutive 30-min clearance studies were carried out.

In the fourth series of experiments (group 4), the effects of cyclooxygenase (COX) synthesis inhibition on renal hemodynamics in nephritic rats (n = 5) were examined. Initially, two consecutive 30-min control clearances were carried out. Then, renal arterial pressure was reduced by ~20 mmHg. A 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. The aortic clamp was released. Indomethacin (5 mg/kg, Sigma) was intravenously administered as a primer, and intravenous solutions were exchanged to an isotonic saline solution containing 1% BSA, 7.5% Inunest, 1.5% PAH, and indomethacin (2 mg·kg⁻¹·h⁻¹) to inhibit prostaglandin synthesis (43). Another 30-min equilibration period was allowed before two consecutive 30-min clearance periods were initiated. Subsequently, the aortic clamp was tightened to further reduce renal arterial pressure by 20 mmHg. Finally, two consecutive 30-min clearance studies were carried out.

At the midpoint of each experimental clearance period, an arterial blood sample (~0.3 ml) was taken. Cells were separated by centrifugation, and plasma was removed. Urine volume was determined gravimetrically. Inulin and PAH concentrations in both plasma and urine were measured by standard spectrophotometry. Sodium concen-
RATION was measured by flame photometry. At the end of the experiment, the rats were killed with large doses of pentobarbital sodium, and the left kidney was removed, decapsulated, blotted dry, and weighed. Then, the kidney was kept in formaldehyde to assess pathology (Fig. 1).

In additional experiments, plasma angiotensin II was determined in five control and five nephritic rats. Surgical procedures were the same as above, except that the abdomen was not opened. After 1 h of equilibration, a 2-ml blood sample was taken from the femoral artery in chilled tubes containing EDTA. Blood samples were centrifuged at 4°C for 10 min. Plasma was kept deep-frozen until RIA assay.

Data are expressed as means ± SE. Statistical analysis was performed using Student’s t-test and analysis of variance followed by a Newman-Keuls test. P < 0.05 was considered statistically significant.

RESULTS

Table 1 summarizes baseline values in control and nephritic rats. Data for control rats (n = 12) were obtained from groups 1 and 2, and those for nephritic rats (n = 22) were taken from groups 1–4. Body weight, mean blood pressure (MBP), and GFR were similar between both groups, whereas RPF was increased in nephritic rats (P < 0.05). In addition, both urine flow (UV) and sodium excretion (UNaV) were elevated in nephritic rats (P < 0.05).

As shown in Fig. 2, RPF (3.82 ± 0.40 to 3.72 ± 0.44 ml·min⁻¹·g kidney wt⁻¹, n = 6) and GFR (0.89 ± 0.08 to 0.89 ± 0.07 ml·min⁻¹·g kidney wt⁻¹) were well autoregulated in control rats with the range of mean arterial pressure (MAP) from 79 ± 1 to 101 ± 1 mmHg. However, in nephritic rats, autoregulation of GFR and RPF disappeared. Following a reduction in MAP from 98 ± 2 to 80 ± 1 mmHg, GFR fell from 0.88 ± 0.05 to 0.75 ± 0.06 ml·min⁻¹·g kidney wt⁻¹ in nephritic rats (n = 6, P < 0.05). Similarly, RPF of nephritic rats was lowered from 4.17 ± 0.63 to 3.20 ± 0.45 ml·min⁻¹·g kidney wt⁻¹ in response to decreases in MAP (P < 0.05).

Infusion of 30% albumin and furosemide increased plasma protein concentration in both control (5.1 ± 0.6 to 8.2 ± 1.1 g/dl, P < 0.01) and nephritic rats (4.9 ± 0.7 to 8.1 ± 1.0 g/dl, P < 0.01). In control rats, the above procedure elevated oncotic pressure from 16 ± 1 to 32 ± 3 mmHg (P < 0.01), calculated according to the equation by Landis and Pappenheimer (21). A similar degree of increments in colloid osmotic pressure (15 ± 2 to 32 ± 2 mmHg, P < 0.01) was observed in nephritic rats. In control rats, lowering MAP from 99 ± 1 to 81 ± 1 mmHg elicited decrements in both GFR (0.87 ± 0.09 to 0.71 ± 0.08 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and RPF (3.52 ± 0.49 to 2.92 ± 0.42 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) during combined infusion of albumin and furosemide. Similarly, in nephritic rats, GFR (0.89 ± 0.07 to 0.75 ± 0.07 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and RPF (3.69 ± 0.78 to 2.99 ± 0.68 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) were decreased following reductions in MAP (101 ± 1 to 80 ± 1 mmHg). As evident in Figs. 3 and 4 (top), the infusion of hyperoncotic solutions with furosemide diminished GFR (−1 ± 1 to −17 ± 2%, P < 0.05) and RPF (−3 ± 2 to −20 ± 3%, P < 0.05) autoregulation in control rats but did not further worsen renal autoregulatory responsiveness in nephritic rats.

In the second series of experiments, control rats showed good autoregulation of GFR and RPF. Thus reductions in MAP from 99 ± 1 to 80 ± 1 mmHg failed to alter either GFR (1.10 ± 0.04 to 1.08 ± 0.05 ml·min⁻¹·g kidney wt⁻¹, n = 6) or RPF (3.50 ± 0.40 to 3.38 ± 0.23 ml·min⁻¹·g kidney wt⁻¹) in control rats. In contrast, lowering MAP from 101 ± 2 to 80 ± 1 mmHg induced decreases in GFR (1.11 ± 0.05 to 0.92 ± 0.08 ml·min⁻¹·g kidney wt⁻¹, n = 6, P < 0.05) and RPF (4.57 ± 0.73 to 3.49 ± 0.27 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) in nephritic rats. Table 2 depicts the effects of 5′-nucleotidase (0.03 U/min) on renal hemodynamics. Although 5′-nucleotidase failed to alter MAP and renal hemody-
nomic and excretory functions in control rats, this enzyme decreased RPF (−34 ± 7%, P < 0.05) and GFR (−22 ± 5%, P < 0.05) of nephritic rats without significant changes in MAP. In addition, 5′-nucleotidase did not significantly alter either UV or UNaV in nephritic rats. During intrarenal infusion of 5′-nucleotidase, autoregulation of GFR (1.05 ± 0.06 to 1.01 ± 0.04 ml·min⁻¹·g kidney wt⁻¹) and RPF (3.25 ± 0.32 to 3.13 ± 0.24 ml·min⁻¹·g kidney wt⁻¹) was well preserved in control rats with the range of MAP from 80 ± 1 to 98 ± 1 mmHg. In the presence of 5′-nucleotidase, reductions in MAP from 101 ± 2 to 80 ± 1 mmHg elicited slight but significant decreases in GFR (0.87 ± 0.06 to 0.81 ± 0.04 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and RPF (2.96 ± 0.41 to 2.75 ± 0.10 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) in nephritic rats. However, as shown in Figs. 3 and 4 (bottom), 5′-nucleotidase did improve the autoregulatory ability of nephritic rats. Administration of 5′-nucleotidase lessened the magnitude of decreases in GFR of nephritic rats associated with blood pressure reduction (−16 ± 5 to −6 ± 2%, P < 0.05). Similarly, 5′-nucleotidase tended to render the degree of pressure-induced changes in RPF of nephritic rats smaller, but it did not attain significance (−16 ± 7 to −6 ± 3%, P = 0.10). In control rats, 5′-nucleotidase did not alter autoregulatory responsiveness of either GFR or RPF.

In complementary studies, although the infusion of 5′-nucleotidase (0.3 U/min) into the renal artery of control rats failed to alter MBP (99 ± 2 to 96 ± 2 mmHg) and GFR (0.96 ± 0.04 to 0.91 ± 0.04 ml·min⁻¹·g kidney wt⁻¹), it decreased RPF (3.77 ± 0.36 to 3.02 ± 0.32 ml·min⁻¹·g kidney wt⁻¹, P < 0.05, n = 5). Although subsequent administration of CPX did not alter MBP (to 99 ± 1 mmHg) or GFR (to 0.97 ± 0.04 ml·min⁻¹·g kidney wt⁻¹), it increased RPF in a dose-dependent manner. At 0.1 mg/kg, CPX tended to reverse decrements in RPF (to 3.43 ± 0.28 ml·min⁻¹·g kidney wt⁻¹). CPX restored RPF (to 3.88 ± 0.24 ml·min⁻¹·g kidney wt⁻¹) at 0.3 mg/kg. These observations support the notion that 5′-nucleotidase elevates renal adenosine levels, activating adenosine-1 receptors.

Because previous investigations indicated that inducible nitric oxide synthase (iNOS) was present within glomeruli of the Thy-1 nephritic model (9), the effects of nitro-L-arginine on renal hemodynamics were examined in nephritic rats. In the present study, the dose of nitro-L-arginine was selected, based on the previous data that it substantially inhibited nitric oxide synthesis and reduced UV and UNaV without changes in the renal autoregulatory capacity of RBF and GFR in normal rats (38). Following the reduction in MAP from 101 ± 2 to 80 ± 1 mmHg, GFR fell from 1.03 ± 0.07 to 0.77 ± 0.03 ml·min⁻¹·g kidney wt⁻¹ in nephritic rats (n = 5, P < 0.05). Similarly, RPF in nephritic rats was lowered from 4.17 ± 0.34 to 3.65 ± 0.24 ml·min⁻¹·g kidney wt⁻¹ (P < 0.05) in response to decreases in MAP. As seen in Table 3, although nitric oxide synthesis inhibition did not alter GFR, nitro-L-arginine significantly reduced RPF in nephritic rats by 27 ± 10%. Of interest, nitro-L-arginine tended to decrease UV and UNaV in nephritic rats, but this did not reach statistical significance. Under nitric oxide synthesis inhibition, lowering MAP from 101 ± 1 to 80 ± 1 mmHg elicited decrements in both GFR (0.96 ± 0.08 to 0.73 ± 0.06 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) and RPF (3.04 ± 0.47 to 2.62 ± 0.39 ml·min⁻¹·g kidney wt⁻¹, P < 0.05) in nephritic rats. As shown in Fig. 5, nitric oxide synthesis inhibition failed to restore the autoregu-
latory ability of GFR and RPF in nephritic rats. At the end of the experiments, MBP reached 118 ± 2 mmHg, following the full release of the aortic clamp.

In the fourth series of studies, the influences of COX inhibition were assessed on renal hemodynamics in nephritic rats, because of recent reports that COX-2 was enhanced in the Thy-1 nephritic model (11). The dose of indomethacin (2 mg·kg<sup>-1</sup>·h<sup>-1</sup>) was used, as it considerably inhibited renal prostaglandin synthesis without alterations of GFR, RPF, and renal autoregulation in normal rats (43). Reductions in MAP from 99 ± 1 to 80 ± 1 mmHg induced decreases in GFR (1.03 ± 0.18 to 0.76 ± 0.04 ml·min<sup>-1</sup>·g kidney wt<sup>-1</sup>, n = 5, P < 0.05) and RPF (4.07 ± 0.30 to 3.35 ± 0.39 ml·min<sup>-1</sup>·g kidney wt<sup>-1</sup>, P < 0.05) in nephritic rats. Table 4 summarizes the influences of COX inhibition on renal circulation in nephritic rats. In nephritic rats, indomethacin elicited decrements in GFR and RPF by 24 ± 9 and 62 ± 10%, respectively. Under COX inhibition, both UV (11.0 ± 1.2 to 4.2 ± 0.3 μl/min, P < 0.05) and U<sub>Na</sub>V (357 ± 72 to 73 ± 4 neu/min, P < 0.05) were diminished in nephritic rats. Even in the presence of indomethacin, reductions in MAP from 100 ± 1 to 80 ± 1 mmHg elicited considerable decreases in GFR (0.69 ± 0.13 to 0.56 ± 0.15 ml·min<sup>-1</sup>·g kidney wt<sup>-1</sup>, P < 0.05) and RPF (1.54 ± 0.22 to 1.29 ± 0.27 ml·min<sup>-1</sup>·g kidney wt<sup>-1</sup>, P < 0.05) in nephritic rats. As shown in Fig. 6, the autoregulatory capacity of GFR and RPF in nephritic rats was not improved by COX inhibition.

Because angiotensin II is a strong positive modulator of TGF (27, 33), and because angiotensin II may also enhance myogenic responsiveness of afferent arterioles (18), we measured plasma angiotensin levels in control and nephritic rats (n = 5 for each group). As shown in Fig. 7, nephritic rats exhibited higher angiotensin II concentrations (32 ± 2 pg/ml) than did control rats (13 ± 2 pg/ml, P < 0.05). It was unlikely that diminished renal autoregulation observed in nephritic rats was attributable to prevailing levels of angiotensin II.

**DISCUSSION**

In contrast to the other organs, the kidney exhibits a remarkable capacity to maintain RBF and GFR constant in the face of marked variations in systemic blood pressure (27). There is consensus that both the myogenic response and TGF are required for efficient renal autoregulation. The former induces afferent arteriolar constriction much faster than the latter (36). Although myogenic responses that are intrinsic to vascular smooth muscle cells are present in various vascular beds including cerebral, mesenteric, and coronary arteries (42), TGF is specific to the kidney (33). Recent analyses of renal autoregulation dynamics reveal that two mechanisms interact with each other (16). Because TGF constricts the terminal portion of the afferent arteriole, it increases upstream pressure, enhancing myogenic constriction and influencing the autoregulatory capacity of adjacent nephrons (17, 37). Furthermore, a TGF mediator induces oscillations in tubular pressure (7, 39). In addition, various factors modulate TGF. Angiotensin II enhances TGF responsiveness (33), but nitric oxide and prostaglandins suppress it (27, 47).

Our previous data demonstrated that an anti-Thy-1 antibody caused mesangiolysis at 1–3 days after injection (29). Using polyclonal antibodies to Thy-1, Iversen et al. (15) reported that, although mesangiolysis did not affect RBF autoregulation, it
did impair GFR autoregulation. In addition, Ren et al. (31) showed that OX-7 inhibited TGF-induced rabbit afferent arteriolar constriction. In the present study, we demonstrated that autoregulation of both GFR and RPF was deranged in nephritic rats. Although the reasons for the discrepancy in observations by Iversen et al. (15) and our group are not clear from the present study, they may relate to differing methods. Iversen et al. (15) showed that mesangiolysis in Wistar rats with 1 mg of the monoclonal antibody 1–22-3. Furthermore, we showed that administration of furosemide and an increase in oncotic pressure diminished GFR and RPF autoregulation in control rats but did not further worsen autoregulation in nephritic rats, providing the evidence that TGF was already spoiled in rats with acute mesangial injury by the anti-Thy-1 antibody. We should emphasize that this was the first in vivo study to assess renal autoregulation in Thy-1 nephritis, which was induced by a strong monoclonal antibody, mAb1–22-3. Indeed, this monoclonal antibody consistently reproduced substantial mesangiolysis (Fig. 1). The present data that RPF was higher in nephritic rats agreed well with previous reports that single-nephron plasma flow was increased in Thy-1 nephritis (48). Yamamoto et al. (48) showed that the ultrafiltration coefficient was reduced in Thy-1 nephritis. Collectively, the present observations indicated that mesangial cell injury occurred during the early course of Thy-1 nephritis and suggest that functioning mesangial cells are required to fully transduce TGF signals from macula densa to afferent arterioles. The present results may shed light on the debate regarding the mediator of TGF. Thomson et al. (45) showed that an inhibition of 5′-nucleotidase with α,β-methylene adenosine 5′-diphosphate did not abolish but diminished TGF responses of single-nephron GFR. Ren et al. (32) reported that this

Table 2. Effects of 5′-nucleotidase (0.03 U/min) on renal hemodynamics

<table>
<thead>
<tr>
<th>Control</th>
<th>Basal</th>
<th>5′-Nucleotidase</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>241±7</td>
<td>98±1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99±1</td>
<td>98±1</td>
</tr>
<tr>
<td>RPF, ml/min g kidney wt⁻¹</td>
<td>3.50±0.40</td>
<td>3.25±0.32</td>
</tr>
<tr>
<td>GFR, ml/min g kidney wt⁻¹</td>
<td>1.10±0.04</td>
<td>1.05±0.06</td>
</tr>
<tr>
<td>UV, µl/min</td>
<td>9.1±1.0</td>
<td>8.5±0.9</td>
</tr>
<tr>
<td>UNaV, neq/min</td>
<td>236±40</td>
<td>219±43</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05.

Table 3. Impact of nitric oxide synthesis inhibition on renal hemodynamics in nephritic rats

<table>
<thead>
<tr>
<th>Thy-1</th>
<th>Basal</th>
<th>Nitro-l-Arginine</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>283±6</td>
<td>101±1</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>101±2</td>
<td>101±2</td>
</tr>
<tr>
<td>RPF, ml/min g kidney wt⁻¹</td>
<td>4.17±0.34</td>
<td>3.04±0.47*</td>
</tr>
<tr>
<td>GFR, ml/min g kidney wt⁻¹</td>
<td>1.03±0.07</td>
<td>0.96±0.08</td>
</tr>
<tr>
<td>UV, µl/min</td>
<td>10.2±1.0</td>
<td>9.9±0.9</td>
</tr>
<tr>
<td>UNaV, neq/min</td>
<td>322±16</td>
<td>307±16</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05.
blocker inhibited TGF-induced afferent arteriolar constriction. Consistent with this, we demonstrated that although intrarenal infusions of 5'-nucleotidase did not completely restore renal autoregulation of nephritic rats, it improved GFR autoregulation. Although the reasons for residual derangements of renal autoregulation with 5'-nucleotidase complement were not clear, it is unlikely that renal insufficiency was involved. In the presence of 5'-nucleotidase, nephritic rats manifested GFR values of 0.87 ± 0.06 ml·min⁻¹·g kidney wt⁻¹. Furthermore, renal autoregulatory capacity was preserved even in remnant kidneys of normotensive WKY rats (2). Although renal insufficiency usually results in renal dysautoregulation, renal autoregulation seems resistant to renal injury in this strain. In addition, 5'-nucleotidase lowered RPF in nephritic rats to the level of control animals, an implication that the doses of this enzyme were physiologically sufficient. Because adenosine downregulates the expression of adenosine-1 receptors (50), adenosine-1 receptors could be upregulated in nephritic rats.

Table 4. Influence of cyclooxygenase inhibition on renal hemodynamics in nephritic rats

<table>
<thead>
<tr>
<th></th>
<th>Thy-1</th>
<th>Basal</th>
<th>indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>251±6</td>
<td>100±1</td>
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</tr>
<tr>
<td>MAP, mmHg</td>
<td>99±1</td>
<td>100±1</td>
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<tr>
<td>RPF, ml/min⁻¹·g kidney wt⁻¹</td>
<td>4.07±0.30</td>
<td>1.54±0.22*</td>
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<tr>
<td>GFR, ml/min⁻¹·g kidney wt⁻¹</td>
<td>1.03±0.18</td>
<td>0.69±0.13*</td>
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<tr>
<td>UV, µl/min</td>
<td>11.0±1.2</td>
<td>4.2±0.3*</td>
<td></td>
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<tr>
<td>U_{Na}V, n eq/min</td>
<td>357±72</td>
<td>73±4*</td>
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Values are means ± SE. *P < 0.05.

Although the possibility remains, it is unlikely that mAb1–22-3 affected myogenic mechanisms, because vascular myocytes do not express the Thy-1 antigen and because adenosine inhibits the afferent arteriolar myogenic response (44). Taken together, the present results are compatible with the findings by Castrop et al. (6) that TGF responses were entirely absent in many but
not all superficial nephrons of ecto-5′-nucleotidase knockout mice and support the notion that 5′-nucleotidase on mesangial cells plays a key role in mediating TGF.

In contrast, Inscho et al. (14) demonstrated that TGF-induced afferent arteriolar constriction was blunted in juxtamedullary nephrons of P2X receptor knockout mice and that an A1 receptor blocker did not alter autoregulatory responses in juxtamedullary nephrons of wild-type mice. We propose that an important physiological mechanism like TGF should be redundant, because TGF works in all nephrons despite nephron heterogeneity. Some nephrons have a small number of mesangial cells that are positioned between the macula densa and afferent arteriole, but the other nephrons assemble a large number of mesangial cell interpositions (Fig. 8). In the latter nephrons, traveling from the macula densa to afferent arteriole should turn ATP into an ample amount of adenosine, thereby inducing afferent arteriolar constriction (10). Alternatively, in the former nephrons, ATP would reach the afferent arteriole before degradation, eliciting afferent arteriolar constriction (46). ATP-induced mesangial membrane depolarization may transduce to afferent arteriolar myocytes (19). This proposal could account for the current observations that nephritic rats with 5′-nucleotidase infusion manifested residual impairments in renal autoregulation.

The present observations demonstrated that 2 days after the administration of mAb1–22-3, nephritic rats exhibited high UV and U_{Na}V. Hirose et al. (11) found that in the Thy-1 nephritic model, renal expressions of phospholipase A2 and COX-2 were enhanced from day 1 and day 4 following anti-Thy-1 antibody injection, respectively (11). In accordance, the present data indicated that COX inhibition with indomethacin substantially decreased UV and U_{Na}V in nephritic rats. Carmines et al. (5) reported that prostaglandin synthesis inhibition elicited moderate antidiuresis and antinatriuresis. Thus the above findings suggest that an augmented renal prostaglandin system in nephritic rats helps excrete more water and sodium as observed in the present study. Of importance, our present data showed that although the administration of indomethacin significantly reduced RPF and GFR in nephritic rats, renal autoregulatory ability was not altered by COX inhibition. Consequently, these findings indicate that prostaglandins appear essential to maintain glomerular circulation in nephritic rats and suggest that modulatory actions of prostaglandins on TGF responsiveness play a small role in determining the renal autoregulatory capacity of nephritic rats.

Because iNOS was enhanced in Thy-1 nephritis (9), there was the possibility that nitric oxide was involved in blunted

Fig. 7. Circulating levels of angiotensin II. Thy-1 indicates nephritic rats. *P < 0.05 vs. control.

Fig. 8. Working hypothesis for mechanisms mediating tubuloglomerular feedback. Pathway 1: ATP released from macula densa should directly stimulate purinergic-2 receptors (P2) on afferent arteriolar myocytes. Pathway 2: ATP activates P2 receptors on mesangial cells, thereby inducing membrane depolarization that could transduce to afferent arteriolar myocytes through gap junctions and subsequently activate voltage-dependent calcium channels. Pathway 3: adenosine formed by ecto-5′-nucleotidase (NT) on mesangial cells would activate adenosine-1 receptors (A1) on afferent arteriolar myocytes.
TGF in nephritic rats (47). However, our data indicated that in nephritic rats, GFR and RPF autoregulation was not ameliorated by nitric oxide synthesis inhibition. In addition, although iNOS is localized in polymorphonuclear leukocytes, which accumulate within 1 h of OX-7 injection, these leukocytes are gone once mesangiolysis is produced (9). It is surprising that nitric oxide synthesis inhibition did not decrease UO or UNaV in nephritic rats. In addition, it reduced RPF but not GFR. In nephritic rats, the phospholipase A2 prostaglandin system seems to replace physiological actions of nitric oxide on tubular transport and vascular tone, accounting for the lack of anti-diuresis, antinatriuresis, and decrement in GFR during nitric oxide synthesis inhibition (38).

Novel findings in the present study constitute the observations that plasma angiotensin II was elevated in nephritic rats. Although angiotensin II considerably constricts afferent arterioles (40), RPF was increased in nephritic rats. Diminished TGF in nephritic rats may contribute to an increase in RPF. Indeed, Ikenaga et al. (13) reported that about half of afferent arteriolar constriction by angiotensin II comes from the enhancement of TGF. Renin release was controlled by various mechanisms, including afferent arteriolar pressure, TGF, and sympathetic nerve activity. Paracrine factors such as angiotensin II, nitric oxide, and prostaglandins are also involved (20). Brown et al. (4) demonstrated that plasma renin was increased in A1 receptor knockout mice. In addition, Yeo et al. (49) reported that ATP itself decreased renin release in juxtaglomerular cells. Thus blunted TGF in nephritic rats could take part in an increase in angiotensin II. Clearly, further studies are required to characterize renin release in nephritic animals.

In summary, the present results implicated that TGF was diminished in Thy-1 nephritic rats, resulting in impaired autoregulation of GFR and RPF. Furthermore, our observations indicated that nephritic rats were diuretic and natriuretic, compared with the control. The present investigations constitute new demonstrations that the renin-angiotensin system is activated in nephritic rats. In addition, our data provided evidence that prostanoids contributed to maintain renal circulation in nephritic rats. Finally, the present findings suggest that mesangial cells and/or 5'-nucleotidase play an important role in mediating renal autoregulation.

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