Urinary excretion of urodilatin is increased during pressure natriuresis in the isolated perfused rat kidney

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Heringlake, Matthias, Klaus Wagner, J an Schumacher, and Horst Pagel. Urinary excretion of urodilatin is increased during pressure natriuresis in the isolated perfused rat kidney. Am. J. Physiol. 277 (Renal Physiol. 46): F347–F351, 1999.—The findings about mechanisms regulating production and excretion of urodilatin [ANP-(95–126)], a member of the atrial natriuretic peptide (ANP) family, are controversial. To elucidate a possible relationship between arterial blood pressure and renal urodilatin excretion, we studied the effects of different perfusion pressures on urine flow (UV), urinary sodium (UNaV), urinary potassium (UKV), and urodilatin excretion (UUROV), and the concentration of urodilatin in the perfusate (P URO) of isolated perfused rat kidneys. Kidneys were perfused for 180 min with constant perfusion pressures (80 and 120 mmHg, respectively; each, n = 4) in a closed circuit system. Samples of urine and perfusate were taken every 30 min. Mean UV, UNaV, UKV, and UUROV values were significantly higher with a perfusion pressure of 120 mmHg than with 80 mmHg, whereas P URO did not change significantly. Serial measurements revealed no direct relation of UUROV with either UNaV or UV. This suggests that renal perfusion pressure is a determinant of UUROV and that urinary and venous effluent concentrations of urodilatin (probably production) are not coupled directly and that UUROV and UNaV may dissociate during acute variations of sodium excretion and UV.

MATERIALS AND METHODS

Experimental Animals

Kidneys were obtained from adult male Sprague-Dawley rats, weighing 200–450 g. The animals had free access to food (diet 1314; Altromin, Lage, Germany) and water up to the experiments.

Preparation of Kidneys

The right kidneys were used for perfusion. The left kidneys were decapsulated and weighed for calculation of the parameters of renal function.

The surgical procedure has been reported previously (19). In brief, after anesthesia (thiopental, 100 mg/kg body wt ip; Byk-Gulden, Constance, Germany) and laparotomy, the right ureter was cannulated by a polyethylene tube. Then, 0.5 ml heparin (200 U/animal iv) was injected, and the right kidney was placed in a copper chamber (37°C). Caudal to the right renal artery, the abdominal aorta was clamped, and a double-barreled cannula was inserted. The mesenteric artery, the left renal artery, and the aorta cranial to the right renal artery were tied up. After the aortic clamp was rapidly opened, perfusion was started in situ (initial flow rate, 10 ml/min). About 4 min later, all connecting structures between the kidney and the organism were cut off, and the animal was removed by lowering the operating table. Recirculation was established in that the renal vein was cannulated by a steel tube, and a copper funnel was placed below the kidney chamber to collect the venous effluent. Thereafter, the perfusion was continued at a constant perfusion pressure (80 or 120 mmHg, respectively).

Perfusion Medium

The perfusion medium was an amino acid- and substrate-enriched Krebs-Henseleit buffer that contained freshly drawn human erythrocytes (hematocrit, 5%) and 60 g/l BSA (Cohn fraction V; Biomol, Hamburg, Germany). BSA was purified by repeated dialysis (three periods of 3 h). The electrolyte and substrate composition of the perfusion medium was (in mM) 140 Na+ 5 K+, 25 HCO3−, 2.5 Ca2+, 1.2 Mg2+, 104 Cl−, 0.72 H

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Sehested et al. (23) investigated patients after uncomplicated cardiac surgery. They observed that urine flow (UV), UUROV, and diastolic blood pressure were positively correlated. These findings may be influenced by the confounding effects of surgery and cardiopulmonary bypass and, therefore, cannot prove that this interaction also applies to general physiology. But in conjunction with the data from Kirchhoff et al. (12), they suggest that UUROV may indeed be influenced by changes in arterial and renal perfusion pressure.

To test this hypothesis, we investigated the effect of different perfusion pressures on renal function parameters, UUROV, and the concentration of urodilatin in the recirculating perfusion solution in an isolated perfused rat kidney model.
PO₄³⁻, 0.18 H₂PO₄, 6 urea, 2 pyruvate, 2 lactate, 1 malate, 8.3 glucose, 1 glutathione, and 1 oxalacetate.

In addition, the perfusion medium contained the following agents: antidiuretic hormone (10 mU/l, Pitressin; Parke-Davis, Munich, Germany), insulin (1 g/l, Inutest; Laevosan, Linz, Austria), gentamicin sulfate (8 mg/l; Beecham, Neuss, Germany), α-tocopherol (15 U/A, Spondyvit; Efeka, Hannover, Germany), ascorbate (10 mg/l), and a complete set of physiological amino acids in a concentration range from 0.5 to 2 mM (Aminoplasmal L-10; Braun, Melsungen, Germany). The perfusion medium was freshly composed and sterile filtered immediately before the experiment.

Experimental Setup

The isolated kidneys were perfused at 37°C in a closed recirculation system. From the reservoir, the perfusion medium (200 ml) was pumped through a dialyzer and regenerated against 5,000 ml dialysate, which was composed like the perfusion medium except that BSA and erythrocytes were omitted. The dialyzer also served for the equilibration (“dialung”) with a prewarmed and moistened gas mixture (5% CO₂-95% O₂). The oxygenated perfusion medium was pumped to the double-barreled aortic cannula, of which the inner part was connected to a pressure transducer (model PR-10–1; Keller, Winterthur, Switzerland). The pressure signal was taken for feedback regulation of the perfusion pump. Perfusion flow and pressure were recorded continuously.

Experimental Design

Two series of experiments were performed. Kidneys were perfused for 3 h with a constant perfusion pressure of 80 and 120 mmHg (n = 4 each). Specimen of urine and perfusate were taken every 30 min. UV was determined gravimetrically. Perfusion flow rate (PFR) was derived from the revolution of the perfusion pump (tacho signal). Glomerular filtration rate (GFR) was calculated on the basis of the clearance of inulin. Sodium and potassium were determined by flame photometry, and urinary sodium (UNaV) and potassium (UKV) were calculated according to standard formula. UROV, the concentration of urodilatin in the perfusion solution (P URO), was determined by a radioimmunnoassay for rat urodilatin (rURO-RIA) by Immundiagnostik (Bensheim, Germany). As previously described by Kirchhoff and Forssmann (13), this antiserum does not cross-react with rat brain natriuretic peptide (rBNP), rat C-type natriuretic peptide (rCNP), or rat atrial natriuretic peptide rANP (99–126). Intra- and interassay variation was 7.4 and 10.3%, respectively. The detection limit was 0.27 pmol/l.

The filtration rate of urodilatin (UROFILT) was calculated as the product of P URO and GFR. Fractional excretion of urodilatin (FE URO) was determined as the ratio of U UROV and UROFILT.

Statistical Analyses

Comparison of groups. The average of six measurements of each variable was calculated. Mean and individual differences between groups were analyzed by Mann-Whitney's test for unpaired observations. All data are presented as median and range.

Time course of individual parameters. Since the number of kidneys in each group was too small for intrapatient nonparametric testing, data were normalized by calculating the relative change to baseline and further analyzed for differences throughout the perfusion period (from 30 min to 180 min) by paired Student's t-test.

The level of statistical significance was set to \( P < 0.05 \).

RESULTS

Comparison of Groups

UV, UNaV, FR Na, UNaV, UROFILT, and U UROV were significantly higher and FR H₂O significantly lower with a perfusion pressure of 120 mmHg than with 80 mmHg (Table 1).

Differences of UV, UNaV, GFR, and U UROV between different pressure groups at individual time points are presented in Fig. 1. The increase of mean GFR with the higher perfusion pressure failed to reach statistical significance (\( P = 0.08 \)), although four of six measurements revealed a significant individual difference (Fig. 1D).

FE URO at 180 tended to be higher with a perfusion pressure of 120 mmHg (Table 2), but this difference was not significant. Average FE URO was not different between both pressure series.

PFR tended to be increased, and, hence, RVR tended to be lower, with higher pressures. But again this difference failed to reach statistical significance (Table 1).

Table 1. Renal function, urinary urodilatin excretion, and urodilatin perfusate concentrations in isolated rat kidneys perfused with different pressures

<table>
<thead>
<tr>
<th>Variable</th>
<th>80 mmHg</th>
<th>120 mmHg</th>
</tr>
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<tbody>
<tr>
<td>UV, ( \mu l \cdot min^{-1} \cdot g^{-1} )</td>
<td>38.6</td>
<td>211.5*</td>
</tr>
<tr>
<td>UNaV, ( \mu mol \cdot min^{-1} \cdot g^{-1} )</td>
<td>3.67</td>
<td>25.4*</td>
</tr>
<tr>
<td>FR Na, %</td>
<td>91.1</td>
<td>78.9*</td>
</tr>
<tr>
<td>UNaV, ( \mu mol \cdot min^{-1} \cdot g^{-1} )</td>
<td>71.6–97.6</td>
<td>71.7–85.4</td>
</tr>
<tr>
<td>GFR, ( \mu l \cdot min^{-1} \cdot g^{-1} )</td>
<td>2.11</td>
<td>6.36*</td>
</tr>
<tr>
<td>PFR, ( ml \cdot min^{-1} \cdot g^{-1} )</td>
<td>198.6–598.8</td>
<td>451.9–875.6</td>
</tr>
<tr>
<td>RVR, ( mmHg \cdot ml \cdot min^{-1} \cdot g^{-1} )</td>
<td>5.93</td>
<td>4.31</td>
</tr>
<tr>
<td>FR H₂O, %</td>
<td>86.7</td>
<td>74.2*</td>
</tr>
<tr>
<td>U UROV, ( \mu mol \cdot min^{-1} \cdot g^{-1} )</td>
<td>30.7</td>
<td>74.6*</td>
</tr>
<tr>
<td>P URO, ( \mu mol/l )</td>
<td>482.7</td>
<td>514.5</td>
</tr>
<tr>
<td>UROFILT, ( \mu mol/l )</td>
<td>419.8–935.2</td>
<td>469.3–721.0</td>
</tr>
<tr>
<td>FE URO, %</td>
<td>19.9</td>
<td>18.7</td>
</tr>
</tbody>
</table>

Values are medians (with ranges in parentheses) and are the average of 6 measurements performed throughout a perfusion period of 180 min (each group, n = 4). UV, urine flow; UNaV, urinary excretion of sodium; FR Na, fractional reabsorption of sodium; UNaV, urinary excretion of potassium; GFR, glomerular filtration rate (inulin clearance); PFR, perfusion flow rate; RVR, renal vascular resistance; FR H₂O, fractional reabsorption of water; U UROV, urinary excretion of urodilatin; P URO, urodilatin perfusate concentration; UROFILT, urodilatin filtration rate; and FE URO, fractional excretion of urodilatin. *\( P < 0.05 \), Mann-Whitney test.
1). P_{uro} did not differ between the respective pressure groups.

**Time Course of Individual Parameters**

Significant variations could only be detected in the high-pressure group. UUROV, FR Na, and FR H_{2}O decreased significantly from \( t_{30} \) to \( t_{180} \) [UUROV, 218.1 \( \text{fmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) (103.0–250.7) to 23.8 \( \text{fmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) (8.3–39.4), \( P < 0.001 \) (Fig. 1C); FR Na, 94.1 (92.2–95.8) to 64.5% (54.4–75.3), \( P < 0.01 \) (not shown); FR H_{2}O, 88.9 (87.1–89.5) to 60.6% (50.2–71.1), \( P < 0.001 \) (not shown)]. UV and UNaV increased during this period [UV, 85.6 (63.5–92.9) to 343.3 \( \mu \text{l} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) (121.5–429.5), \( P < 0.05 \) (Fig. 1A); UNaV, 16.8 (4.7–7.9) to 45.3 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} \) (15.8–59.4), \( P < 0.05 \) (Fig. 1B)].

**DISCUSSION**

An increase of urodilatin excretion was observed if renal perfusion pressure was raised from 80 to 120 mmHg. This increase of urodilatin excretion was accompanied by increased UV, UNaV, and U K_{V}, decreased

**Table 2. Time course of filtered urodilatin and fractional excretion of urodilatin in isolated rat kidneys during 180 min of perfusion with different pressures**

<table>
<thead>
<tr>
<th></th>
<th>30 min (median) (range)</th>
<th>60 min (median) (range)</th>
<th>90 min (median) (range)</th>
<th>120 min (median) (range)</th>
<th>150 min (median) (range)</th>
<th>180 min (median) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UROFILT, ( \text{fmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} )</td>
<td></td>
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<tr>
<td>80 mmHg</td>
<td>233.4 (41.4–293.2)</td>
<td>158.0 (120.2–266.1)</td>
<td>237.2 (71.1–332.6)</td>
<td>222.1 (55.7–336.3)</td>
<td>224.5 (75.6–312.4)</td>
<td>184.6 (115.3–309.3)</td>
</tr>
<tr>
<td>120 mmHg</td>
<td>312.8* (310.5–576.8)</td>
<td>618.6* (346.1–702.2)</td>
<td>352.9 (286.1–682.0)</td>
<td>352.6 (196.7–503.9)</td>
<td>314.9 (188.5–644.3)</td>
<td>371.5 (206–479.8)</td>
</tr>
<tr>
<td>FE_{uro}, %</td>
<td>80 mmHg</td>
<td>31 (4–70)</td>
<td>24 (6–40)</td>
<td>1 (7–49)</td>
<td>1 (4–60)</td>
<td>1 (3–46)</td>
</tr>
<tr>
<td></td>
<td>120 mmHg</td>
<td>54 (33–81)</td>
<td>17 (14–23)</td>
<td>17 (5–41)</td>
<td>7 (4–16)</td>
<td>9 (2–11)</td>
</tr>
</tbody>
</table>

Values are medians (with ranges in parentheses). * Significant difference (\( P < 0.05 \)) between renal perfusion pressure groups at respective time points (Mann-Whitney test). † Significant difference (\( P < 0.01 \)) in comparison with baseline at \( t_{30} \) (Student’s t-test).
water reabsorption, and a tendency of increased GFR. In contrast, no variations of urodilatin perfusate concentration with different perfusion pressures were detected.

Urodilatin effects on kidney function are mediated by natriuretic peptide receptors A and B (25), which are also binding sites for ANP. Although several studies clearly demonstrate, that urodilatin, if applied exogenously, is a more potent natriuretic peptide than ANP (11), it is still controversial as to which factors regulate the production and excretion of this hormone.

Drummer et al. (3) demonstrated that $U_{Na}$ and $U_{uroV}$ follow a comparable circadian course and that $U_{uroV}$ is increased after an infusion of saline in healthy volunteers. Meyer et al. (17) reported that urodilatin excretion is concomitantly increased with higher nutritional sodium load in humans. These studies suggest that $U_{uroV}$ is influenced by changes of sodium load and the plasma concentration of sodium. Comparable data were presented by Emmeluth and coworkers (5), who observed an increase of urodilatin excretion accompanied by a strong natriuresis in dogs during cerebral split-infusion of hypertonic saline. Interestingly, these effects on renal function were also detectable, if the kidneys were denervated (4), suggesting an additional "factor" transmitting the increase of cerebral sodium concentration to the kidneys. Goetz et al. (7) observed an increase of urodilatin excretion and natriuresis in conscious dogs after inflation of a balloon in the left atrium, suggesting that cardiopulmonary receptors and atrial stretch are involved in the regulation of urodilatin excretion. Interestingly, this effect was no longer detectable if cardiac nerves had been cut.

As stated above, several studies indicate that arterial blood pressure may be another important determinant of urodilatin excretion (12, 23) and its diuretic effects. But evidence supporting this concept is still indirect. With respect to the difficulties to achieve constant sodium, volume, and pressure conditions in intact animals, we choose to test the effects of different perfusion pressures on urinary urodilatin excretion and production, with the latter estimated from the concentration of urodilatin in the perfusion solution, in isolated perfused rat kidneys.

We were able to show pressure dependency of $U_{uroV}$, an important implication. Therefore data from studies reporting alterations of urodilatin excretion during specific experiments should be interpreted with regard to possible interactions of the investigated variable and blood pressure changes. This is exemplified by the study of Drummer and coworkers (3) on the effects of an acute infusion of saline on $U_{uroV}$. Though an infusion of 2 liters of saline within 25 min will usually also affect blood pressure, this work does not present cardiovascular data.

Although the observed changes of renal function parameters between the 80 and 120 mmHg series are well-known effects of increased renal perfusion pressure (14), the concomitant increase of urodilatin excretion, UV, and sodium excretion with higher pressures and the unchanged concentration of urodilatin in the perfusate solution requires further comments.

The increased $U_{uroV}$ with higher perfusion pressure was accompanied by alterations of renal function parameters, which have been implicated in the effects of exogenously applied urodilatin, i.e., UV, $U_{Na}$, and GFR (8, 9, 18). The concomitant increase of UV, $U_{Na}$, and $U_{uroV}$ is suggestive that, in addition to several other factors (14), urodilatin might be involved in the renal adaptations to changes in blood pressure. The lack of an effect of higher perfusion pressure on mean GFR must be interpreted with caution, since four of six measurements comparing individual GFR values in the high- and low-pressure group were indeed significantly different.

Interestingly, we detected comparable urodilatin concentrations in the perfusate within different pressure groups. This demonstrates that low concentrations of urodilatin are present in the venous effluent; an observation that is in accordance with a recent study reporting that blood urodilatin concentrations in humans are in the range of 9 to 12 pg/ml (26). Additionally, if the concentration of urodilatin in the venous effluent is an estimate of basal urodilatin production, then our data might suggest that urodilatin production and urinary output are not directly coupled and that urodilatin production is not influenced by increases of renal perfusion pressure.

The increase of UV and $U_{Na}$ during longer perfusion periods is a well-recognized phenomenon in isolated kidneys (16), probably due to "washout" of medullary hypertonicity. Measures to reduce washout are the addition of erythrocytes and antidiuretic hormone to the perfusion medium. The addition of erythrocytes has been demonstrated to stabilize organ function significantly (24); for unknown reasons this can be accomplished even with a hematocrit of 5% (20). Low concentrations of antidiuretic hormone have been shown to improve medullary concentration (15). This may be explained by a moderate increase of RVR and a reduction of the hyperperfusion of medullary vessels observed in the nearly unconstricted ex vivo preparation (2).

Further natriuretic stability can be achieved by increasing the albumin concentration in the perfusate solution to 75 g/l albumin or more. Unfortunately, this is accompanied by a decrease of GFR and altered glomerular dynamics (16). Recently, we demonstrated that urodilatin increases GFR in isolated rat kidneys perfused with 60 g/l albumin (10), whereas Kirchhoff et al. (12) did not observe such an effect with higher perfusate concentrations (100 g/l) of BSA. Therefore we chose a concentration of 60 g/l albumin for this experiments as a compromise between preserved glomerular vessel reactivity and slight natriuretic instability.

We observed a different pattern of UV, $U_{Na}$, and $U_{uroV}$, respectively, during the perfusion period. If the increase of $U_{uroV}$ with higher perfusion pressure and consequently higher UV and $U_{Na}$ was induced by the increase of tubular flow, then one would expect that $U_{uroV}$ is also increased with UV and $U_{Na}$ during washout induced diuresis and natriuresis.

In contrast, we observed a decrease of $U_{uroV}$ under these circumstances, while $P_{uro}$ remained unchanged. This suggests that $U_{uroV}$ is not increased in parallel with UV and that it is unlikely that the observed
pressure-dependent increase of \( U_{UROV} \) is an effect of the increased diuresis per se. \( UROFILT \) was significantly higher in the 120 mmHg pressure series than with a perfusion pressure of 80 mmHg. Hence the increase of \( U_{UROV} \) in the high-pressure group might be an effect of the increased urodilatin filtration rate. But the different course of \( FE_{URO} \) and \( UROFILT \) throughout the perfusion period makes such an explanation unlikely. Even if \( FE_{URO} \) at \( t=30 \) was not significantly higher in the 120 mmHg series than in the low-pressure group, an immediate decrease of this parameter was observed at \( t=60 \) and \( UROFILT \) remained low until the end of the experiment. A qualitatively comparable time course and a nearly statistically significant decrease of \( FE_{URO} \) was observed in the 80 mmHg series. In contrast to these observations, \( UROFILT \) remained nearly constant from \( t=30 \) to \( t=180 \) in both groups. Hence it is not likely that recirculation of urodilatin and an increase of \( UROFILT \) due to a relative increase of GFR may account for the increase of \( U_{UROV} \) with higher perfusion pressure.

The low \( U_{UROV} \) from \( t=120 \) to \( t=180 \), when UV and natriuresis were maximal, deserves further comment. This dissociation of \( U_{UROV} \) and either UV or \( U_{NaV} \) suggests that \( U_{UROV} \) and \( U_{NaV} \) are not directly correlated, at least during acute variations of UV and sodium excretion. Seemingly, such a dissociation may lead one to question the primary role of urodilatin in the regulation of natriuresis; however, it may also be explained as a counterregulatory decrease of \( U_{UROV} \) due to an increase of tubular sodium. This question must be answered in further studies.

It is concluded that renal perfusion pressure and, hence, arterial blood pressure are determinants of \( U_{UROV} \) and that urinary and venous effluent concentrations of urodilatin, probably an estimate of urodilatin production, are not coupled directly. These findings may have important implications for further studies investigating the regulation of urodilatin excretion.

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