Diuresis and Natriuresis Caused by Activation of VR1-Positive Sensory Nerves in Renal Pelvis of Rats

Yi Zhu, Youping Wang, Donna H. Wang

Abstract—To test the hypothesis that activation of the vanilloid receptor 1 (VR1) expressed in sensory nerves innervating the renal pelvis leads to diuresis and natriuresis, a selective VR1 receptor agonist, capsaicin (2.4 nmol), or vehicle was perfused intravenously or into the left renal pelvis of anesthetized rats at a rate without changing renal perfusion pressure. Mean arterial pressure was not altered by capsaicin administered intravenously or into the renal pelvis. Capsaicin perfusion into the left renal pelvis but not intravenously caused significant increases in urine flow rate and urinary sodium excretion bilaterally in a dose-dependent manner, which were abolished by capsazepine, a selective VR1 receptor antagonist, given ipsilaterally to the renal pelvis or by ipsilateral renal denervation. Capsaicin given intravenously or into the left renal pelvis increased plasma calcitonin gene–related peptide levels to the same extent. Increased plasma calcitonin gene–related peptide levels induced by capsaicin (68.9±2.8 pg/mL) perfusion into the renal pelvis was prevented either by capsazepine (22.5±10.1 pg/mL) given ipsilaterally into the renal pelvis or by ipsilateral renal denervation. Taken together, our data show that unilateral activation of VR1-positive sensory nerves innervating the renal pelvis leads to bilateral diuresis and natriuresis via a mechanism that is independent of plasma calcitonin gene–related peptide levels. These data suggest that VR1-positive sensory nerves in the kidney enhance renal excretory function, a mechanism that may be critically involved in sodium and fluid homeostasis.

Key Words: natriuresis ■ diuresis ■ sensory nerves ■ vanilloid receptor 1 ■ capsaicin ■ renal pelvis

The renal pelvis is heavily innervated by primary sensory afferent nerves with their nerve terminals distributing in mucous and in proximity to the epithelial cells. The renal pelvis therefore is considered as a sensory organ sensing mechano-stimuli and chemo-stimuli leading to altered renal excretory function such as diuresis and natriuresis. Previous studies have shown that ipsilateral obstruction to urine flow or elevation of pressure in the renal pelvis activates renal mechano-sensitive neurons, which results in an increase in ipsilateral afferent renal nerve activity (ARNA). Increased ipsilateral ARNA subsequently induces a contralateral diuresis and natriuresis via an inhibition on contralateral efferent renal nerve activity, which is known as the renorenal reflex.

The vanilloid receptor (VR1), also known as the capsaicin receptor or a member of the transient receptor potential vanilloid subfamily, was cloned by the use of the “hot” pepper-derived vanilloid compound, capsaicin, as a ligand. The VR1 receptor, a ligand gate ion channel, is mainly expressed in a subpopulation of primary sensory afferent fibers with neuroselectivity, the VR1 has been suggested to play a role in regulating vasodilatation in a variety of vascular beds, salt-sensitive hypertension, bladder hyper-reflexia, and respiratory diseases.

A subpopulation of sensory nerves innervating the kidney expresses VR1 receptors. It has been shown that perfusion of capsaicin into the renal pelvis increases ipsilateral ARNA, suggesting that the renal pelvis is innervated by VR1-positive sensory nerves capable of sensing stimuli imposed on the renal pelvis. However, many questions remain to be answered. First, capsaicin is an irritant, which may cause VR1-independent nonspecific effects. It is unknown whether capsaicin-induced increases in ARNA are VR1-specific/VR1-dependent effects. Second, although it is known that intravenous injection of capsaicin causes an increase in plasma levels of sensory neuropeptides such as calcitonin gene–related peptide (CGRP) or substance P, it is unknown whether renal pelvic perfusion of capsaicin is capable of inducing a systemic increase in these neuropeptide levels, and if so, whether increased circulating levels of these neuropeptides contribute to capsaicin action. Third, although capsaicin increases ipsilateral ARNA activity, renal excretory function in the ipsilateral as well as contralateral kidneys is unknown. Therefore, the present study was designed to test the hypo-
esis that activation of the VR1 receptor via perfusion of capsaicin into the renal pelvis contributes to diuresis and natriuresis of bilateral kidneys via an ipsilateral and contralateral renorenal reflex. Capsaicin was given either intravenously or into the unilateral renal pelvis in the presence or absence of a selective VR1 receptor antagonist or unilateral renal denervation. Plasma CGRP levels and renal excretory function in the both kidneys were examined.

**Methods**

**Animal Groups and Protocols**

All experiments were approved by the Institutional Animal Care and Use Committee. Male Wistar rats weighing 312±5 grams (Charles River Laboratories, Wilmington, Mass) were housed in the animal facility 1 week before the experiment. Rats were divided into 7 groups and subjected to the following treatments: (1) control (vehicle), 5% ethanol, and 5% tween 80 in saline given via left renal pelvis perfusion (LRPP) (vehicle LRPP, n=5); (2) capsaicin (CAP), a selective VR1 receptor agonist, given at 2.4 nmol intravenously (CAP intravenous, n=5); (3) CAP at 2.4 nmol given via LRPP (CAP LRPP, n=5); (4) capsazepine (CAPZ), a selective VR1 receptor antagonist, given at 24 nmol via LRPP before CAP perfusion (CAPZ-CAP LRPP, n=6); (5) CAP given at 24 nmol via LRPP (CAPZ LRPP, n=5); (6) acute left renal denervation (RD) before CAP perfusion via LRPP (RD-CAP LRPP, n=5); and (7) acute left RD before vehicle LRPP (RD-vehicle LRPP, n=5). An additional 4 groups of rats (n=5 in each) were used for determining the effect of vehicle or CAP on renal excretory function at doses of 0.04, 0.4, and 2.4 nmol given via LRPP.

All rats were intraperitoneally administered pentobarbital sodium at 50 mg/kg and maintained with an intravenous infusion at 10 mg/kg per hour at 50 μL/min. Catheters were placed in the right jugular vein for administration of drugs and in the right carotid artery for monitoring mean arterial pressure (MAP) with a Statham 231D pressure transducer coupled to a Gould 2400s recorder (Gould Instrument Systems, Valley View, Ohio). Polyethylene (PE-50) catheters were inserted into both of the ureters via a midriff incision. A fine outlet tube of MD-2000 (ID 0.18/OD 0.22 mm; BASI, West Lafayette, Ind) was placed inside the PE-50 catheter, with its tip in the renal pelvis during a 3-minute perfusion of drug at the rate of 20 μL/min that did not change renal pelvis pressure.2

The experiments started ~1.5 hours after the end of the surgery. LRPP consisted of 2 3-minute segments, ie, CAPZ perfused within the first 3-minute segment, and CAP within the second 3-minute segment. In the case when CAP or CAPZ was perfused alone, the other segment was perfused with vehicle. In controls, vehicle was perfused in both segments without perfusion of CAP or CAPZ. Urine samples were collected for 10 minutes before and after each experiment protocol for analyses of urine flow rate (Uflow) and urinary sodium excretion (UNa). Urinary sodium excretion was measured using a flame photometer (model IL-943; Instrumentation Laboratory). At the end of experiment, blood samples were collected for determining plasma CGRP levels.

**Radioimmunoassay**

A rabbit anti-rat CGRP radioimmunoassay kit (Peninsula Laboratories Inc, San Carlos, Calif) was used to determine the CGRP content in plasma. This antibody has 100% cross-reactivity with rat α-CGRP and 79% with rat β-CGRP. There is no cross-reactivity with rat amylin, calcitonin, somatostatin, or substance P.

**Verification of Acute Renal Denervation**

The left kidney was denervated by transecting left renal nerves and painting the renal artery with 10% phenol in absolute ethanol. Thereafter, a bipolar stimulating electrode was placed on the left lumbar sympathetic chain above the left kidney. A flexible fiberoptic probe was placed into the renal cortex and connected to a laser Doppler flowmeter (Periflux System 5000; Perimed) for monitoring

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>Treatment</th>
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</thead>
<tbody>
<tr>
<td>Vehicle LRPP</td>
<td>129±7</td>
<td>132±6</td>
</tr>
<tr>
<td>CAP intravenous</td>
<td>131±5</td>
<td>133±5</td>
</tr>
<tr>
<td>CAP LRPP</td>
<td>123±7</td>
<td>125±4</td>
</tr>
<tr>
<td>CAPZ+CAP LRPP</td>
<td>122±4</td>
<td>122±4</td>
</tr>
<tr>
<td>CAPZ LRPP</td>
<td>122±7</td>
<td>124±8</td>
</tr>
<tr>
<td>RD-CAP LRPP</td>
<td>125±6</td>
<td>127±5</td>
</tr>
<tr>
<td>RD-vehicle LRPP</td>
<td>118±8</td>
<td>122±7</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SE, n=5–6 rats in each group. CAP indicates capsaicin; LRPP, left renal pelvis perfusion; CAPZ, capsazepine; RD, renal denervation.

**Results**

**CAP and Dose–Response Curve**

CAP given at doses of 0.04, 0.4, and 2.4 nmol into the left renal pelvis significantly increased ipsilateral and contralateral Uflow (Figure 1a and 1b) and UNa (Figure 1c and 1d) of kidneys in a dose-dependent fashion. No difference existed between baselines of control and different CAP-treated groups. Uflow (ipsilateral: 5.4±0.2 versus 7.9±0.7, 9.9±0.7, 15.7±2.4; and contralateral: 5.5±0.2 versus 7.5±0.6, 9.4±0.6, 12.8±1.5 μL/min per gram; P<0.05) and UNa (ipsilateral: 0.7±0.1 versus 1.1±0.1, 1.4±0.1, 1.9±0.2; and contralateral: 0.6±0.1 versus 1.0±0.1, 1.4±0.2, 2.2±0.3 μmol/min per gram; P<0.05) were increased to a similar degree in both kidneys in response to the same dose of CAP perfusion into the left renal pelvis.

**Drugs**

Capsaicin (Sigma) was dissolved in ethanol (5% v/v), tween 80 (5% v/v), and saline to make a stock solution of 65 nmol/μL, and was diluted in saline for intravenous and renal pelvis perfusion. Capsazepine (Calbiochem, San Diego, Calif) was dissolved in DMSO (10% v/v), tween 80 (10% v/v), and saline to make a stock solution of 53 nmol/μL, and was diluted in saline for renal pelvis perfusion.

**Statistical Analysis**

All values were expressed as means±SE. The differences among groups were analyzed using 1-way ANOVA followed by the Tukey-Kramer multiple comparison tests. The time course of Uflow after acute left renal denervation was analyzed using 2-way ANOVA with repeated measures for 1 factor. Comparisons of MAP before and after administration of drugs and rCBF before and after acute renal denervation were performed by the use of a paired t test. Differences were considered statistically significant at P<0.05.
Effect of CAPZ on CAP-Induced Diuresis and Natriuresis

CAP given at 2.4 nmol into the left renal pelvis caused significant increases in bilateral Uflow (ipsilateral: 6.8±1.3 versus 15.8±3.1; and contralateral: 6.3±1.0 versus 12.6±1.9 µL/min/g; P<0.05). Whereas CAPZ given alone at 24 nmol into the left renal pelvis had no effect, this dose of CAPZ abolished CAP-induced increases in Uflow (ipsilateral, CAP 15.8±3.1 versus CAPZ-CAP 5.9±1.4; contralateral, CAP 12.6±1.9 versus CAPZ-CAP 5.4±1.3 µL/min per gram; P<0.05) and UNa (ipsilateral, CAP 2.0±0.5 versus CAPZ-CAP 0.8±0.3; contralateral CAP 1.7±0.4 versus CAPZ-CAP 0.6±0.3 nmol/min per gram; P<0.05) of both kidneys.

Effect of RD on rCBF

Acute left renal denervation did not alter MAP (before RD, 125±6; and after RD, 127±5 mm Hg; P>0.05). The ipsilateral Uflow was significantly greater than that of the contralateral side after acute left renal denervation (Figure 3), but there was no significant difference between Uflows of both kidneys before starting experimental protocols (ipsilateral 6.5±0.9 versus contralateral 4.7±0.8 µL/min per gram; P>0.05). Stimulation of the left lumbar sympathetic chain induced marked decreases in renal cortical blood flow (14.7±0.6 periflux unit) in kidneys with intact renal innervation but had little effect (1.7±1.1 periflux unit) in kidneys with acute renal denervation (Figure 4) (P<0.01).

Effect of RD on CAP-Induced Diuresis and Natriuresis

Whereas left renal denervation combined with vehicle perfusion did not alter bilateral Uflow and UNa of kidneys, left renal denervation abolished bilateral increases in Uflow (ipsilateral: CAP 15.8±3.1 versus RD+CAP 5.7±0.7; and contralateral, CAP 12.6±1.9 versus RD+CAP 4.6±0.4 µL/min per gram; P<0.05) and urine sodium excretion (ipsilateral, CAP 2.0±0.5 versus RD+CAP 0.7±0.1; and contralateral, CAP 1.7±0.4 versus RD+CAP 0.5±0.1 µmol/min per gram; P<0.05) of kidneys induced by CAP given into the left renal pelvis (Figure 2).

Analysis of Plasma CGRP Levels

Administration of CAP at the same dose intravenously or into the left renal pelvis markedly increased plasma CGRP levels (vehicle, 29.7±3.0; CAP intravenous, 68.2±11.1; CAP LRPP, 68.9±2.8 pg/mL; P<0.01). The increases in plasma CGRP levels induced by CAP given into the renal pelvis were abolished either by ipsilateral blockade of the VR1 receptor with CAPZ (22.5±10.1 pg/mL) or by ipsilateral acute renal denervation (25.9±2.3 pg/mL; Figure 5).

Discussion

The aim of the present study was to test the hypothesis that activation of the VR1 receptor via perfusion of CAP into the renal pelvis contributes to diuresis and natriuresis of bilateral kidneys via an ipsilateral and contralateral renorenal reflex and to determine the possible mechanisms mediating the effect of VR1 activation. Although it has been previously shown that perfusion of CAP into the renal pelvis increases ipsilateral ARNA, several uncertainties exist. CAP is an
irritant, and it is unknown whether CAP-induced increases in ARNA are VR1-specific/VR1-dependent effects. Moreover, it is unknown whether renal pelvic perfusion of CAP is capable of inducing a systemic increase in sensory neuropeptide levels, and if so, whether increased circulating levels of these neuropeptides contribute to CAP action. Finally, it is unknown whether ipsilateral CAP perfusion into the renal pelvis affects renal excretory function in the ipsilateral as well as contralateral kidneys. Our results show: (1) activation of the VR1 by CAP given into the renal pelvis unilaterally but not intravenously increases Uflow and UNa bilaterally in a dose-dependent fashion without changing blood pressure; (2) the increases in Uflow and UNa in both kidneys induced by activation of the VR1 expressed in sensory nerves innervating the unilateral renal pelvis are abolished either by ipsilateral blockade of the VR1 receptor or by ipsilateral renal denervation; (3) activation of the VR1 by CAP given intravenously or into the unilateral renal pelvis increases plasma CGRP levels; and (4) the increased plasma CGRP levels by CAP perfusion into the renal pelvis are abolished either by ipsilateral blockade of the VR1 receptor or by ipsilateral renal denervation. Taken together, these data indicate for the first time that the VR1 receptors expressed in sensory nerves innervating the renal pelvis play an important role in mediating renal excretory function via an ipsilateral and contralateral renorenal reflex.
In the present study, perfusion of CAP at a much lower dose (2.4 nmol) than these previous studies either intravenously or into the renal pelvis, renal excretory function was not altered. Whereas CAP at this lower dose given intravenously had no effect on Uflow or UNa, this dose of CAP given into the renal pelvis unilaterally increased bilateral Uflow and UNa, suggesting only local renal administration of CAP was efficient. Second, ipsilateral blockade of the VR1 or ipsilateral renal denervation abolished CAP-induced increases in Uflow and UNa of both kidneys and CAP-induced increases in plasma CGRP levels (CAP LRPP, 68.9±2.8 pg/mL; CAPZ+CAP LRPP, 22.5±10.1; RD+CAP LRPP, 25.9±2.3 pg/mL), indicating that enhancement of contralateral renal function was caused by renorenal reflex rather than direct activation of VR1 in the contralateral renal pelvis by increasing circulating CAP levels. Finally, our unpublished data showed that administration of $^{125}$I-α-CGRP into the left renal pelvis resulted in almost undetectable plasma but high urine $^{125}$I-α-CGRP levels. In contrast, the same amount of $^{125}$I-α-CGRP given intravenously led to a plasma level that was ≈30-times higher than that given into the renal pelvis. These data provide strong support for the notion that enhanced bilateral renal function is the result of renorenal reflex caused by activation of the VR1 expressed in the unilateral renal pelvis.

Renal nerves consist of both sensory afferent and sympathetic efferent fibers that distribute in kidneys. Renal denervation by transecting renal nerves and painting the renal artery with 10% phenol destroys both sensory afferent and sympathetic efferent nerves. It has been shown that renal blood flow in kidneys with intact innervation is significantly decreased when ipsilateral lumbar sympathetic chain is stimulated with conventional nerve stimulation, but such decrease was absent (<5% change) when the kidney is denervated. In the present study, perfusion of CAP intravenously or into the renal pelvis increased plasma CGRP levels to the same extent (∼20 pmol/L) without changing blood pressure. Therefore, increased plasma CGRP levels induced by the dose of CAP given in this study did not reach the circulating concentration of CGRP that could induce hemodynamic changes. Although plasma CGRP levels were increased by CAP given intravenously or into the renal pelvis, renal excretory function was enhanced only by CAP perfusion into the renal pelvis, suggesting enhanced renal excretory function is independent of the elevation in plasma CGRP levels. Whereas changes in plasma CGRP levels dissociated with that of renal function, it has been shown that activation of the substance P receptor in the renal pelvis plays an essential role in contralateral inhibitory renorenal reflex. In addition, CGRP has been suggested to enhance the response of afferent renal nerve activity to increased renal pelvic pressure by retarding substance P metabolism. Therefore, we cannot rule out the possibility that local tissue neuropeptides released from VR1-positive sensory nerves interact and synergistically play a role in mediating CAP-induced increases in diuresis and natriuresis.

The fact that CAP perfusion into the unilateral renal pelvis leads to increases in Uflow and UNa bilaterally raises the possibility that CAP may enter into the circulation and may activate VR1 expressed in sensory nerves innervating both kidneys. However, evidence generated from the present study indicates that this is unlikely. First, activation of the VR1 by CAP given into the renal pelvis bilaterally but not intravenously increased Uflow and UNa, suggesting only local renal administration of CAP was efficient. Second, ipsilateral blockade of the VR1 or ipsilateral renal denervation abolished CAP-induced increases in Uflow and UNa of both kidneys and CAP-induced increases in plasma CGRP levels (CAP LRPP, 68.9±2.8 pg/mL; CAPZ+CAP LRPP, 22.5±10.1; RD+CAP LRPP, 25.9±2.3 pg/mL), indicating that enhancement of contralateral renal function was caused by renorenal reflex rather than direct activation of VR1 in the contralateral renal pelvis by increasing circulating CAP levels. Finally, our unpublished data showed that administration of $^{125}$I-α-CGRP into the left renal pelvis resulted in almost undetectable plasma but high urine $^{125}$I-α-CGRP levels. In contrast, the same amount of $^{125}$I-α-CGRP given intravenously led to a plasma level that was ≈30-times higher than that given into the renal pelvis. These data provide strong support for the notion that enhanced bilateral renal function is the result of renorenal reflex caused by activation of the VR1 expressed in the unilateral renal pelvis.
feature may represent harmonization of the nerve network in maintaining and stabilizing biologic homeostasis.

In conclusion, the results of the present study show that unilateral activation of the VR1 in the renal pelvis leads to bilateral diuresis and natriuresis, indicating that the VR1 receptor expressed in sensory nerves innervating the renal pelvis plays an important role in modulating renal excretory function. These effects of VR1 are independent of changes in blood pressure or circulating levels of CGRP.

Perspectives

It is well-known that mechano-receptors and chemo-receptors in the renal pelvis monitor and sense pathophysiologic changes in the renal interstitium. Increases in intrarenal pressure caused by obstruction to urine flow or changes of intrarenal chemical environment caused by pathophysiologic conditions such as ischemia or inflammation may activate primary sensory nerves, leading to modulation of renal excretory function. Moreover, heart failure and hypertension may involve unbalance of sodium and water homeostasis. Although it has been shown that endogenous renal pelvic substance P rather than CGRP directly mediates the renorenal reflex in the face of increased renal pelvic pressure or ion concentrations, evidence showed that CGRP and substance P do interact such that the former activates the receptor of the latter in the renal pelvis by decreasing its metabolism.23 Our results indicate that altered VR1 expression or function in the renal pelvis may alter renal excretory function via a CGRP and/or substance P–dependent pathway, a mechanism intimately involved in the regulation of sodium and water homeostasis in health and disease.

Acknowledgments

This work was supported in part by National Institutes of Health (grants HL-57853 and HL-73287) and a grant from Michigan Economic Development Corporation. Dr Wang is an American Heart Association Established Investigator.

References

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Hypertension. 2005;46:992-997; originally published online August 8, 2005;
doi: 10.1161/01.HYP.0000174603.27383.67
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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