Impaired responsiveness of renal mechanosensory nerves in heart failure: role of endogenous angiotensin

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Kopp, Ulla C., Michael Z. Cicha, and Lori A. Smith. Impaired responsiveness of renal mechanosensory nerves in heart failure: role of endogenous angiotensin. Am J Physiol Regul Integr Comp Physiol 284: R116–R124, 2003. First published September 12, 2002; 10.1152/ajpregu.00336.2002.—Increasing renal pelvic pressure results in PGE2-mediated release of substance P. Substance P increases afferent renal nerve activity (ARNA), which leads to a reflex increase in urinary sodium excretion (UNaV). Endogenous ANG II modulates the responsiveness of renal mechanosensory nerves. The ARNA and UNaV responses are suppressed by low- and enhanced by high-sodium diet. We examined whether the ARNA responses are altered in rats with congestive heart failure (CHF), a condition characterized by increased ANG II and sodium retention. The ARNA responses to increasing renal pelvic pressure ≤7.5 mmHg were suppressed in CHF vs. sham-CHF rats fed normal sodium diet. In CHF rats, increasing renal pelvic pressure 2.5 and 7.5 mmHg increased ARNA 0 ± 1 and 13 ± 2% (P < 0.01) before and 9 ± 1 (P < 0.01) and 19 ± 1% (P < 0.01) during renal pelvic perfusion with losartan. Losartan had no effect on the ARNA responses in sham-CHF rats. In isolated renal pelvises from CHF rats, PGE2 increased substance P release from 11 ± 2 to 15 ± 3 pg/min (not significant) without and from 16 ± 2 to 30 ± 4 pg/min (P < 0.01) with losartan in the incubation bath. Losartan had no effect on PGE2-mediated substance P release in sham-CHF rats. In conclusion, the responsiveness of renal mechanosensory nerves is impaired in CHF rats due to ANG II inhibiting PGE2-mediated release of substance P from renal pelvic nerves.

Immuno histochemical studies have localized the majority of the substance P-containing sensory nerves in the kidney to the renal pelvic wall (25, 33, 48). Activation of these sensory nerves by increased renal pelvic pressure involves bradykinin activating bradykinin-2 receptors with a resultant activation of the phosphoinositide system and cyclooxygenase-2 (COX-2) (24, 26, 31). Activation of COX-2 leads to increased PGE2 synthesis in the renal pelvic wall (24, 27, 28). PGE2 causes a calcium-dependent release of substance P via activation of the cAMP-PKA transduction pathway (20, 23). Substance P increases ARNA by activating substance P receptors in the renal pelvic area (5, 26, 28, 30).

The natriuretic nature of the renal reflexes implies that activation of these reflexes may contribute to the spectrum of renal mechanisms involved in the renal control of water and sodium homeostasis. This theory is supported by our previous studies showing that the responsiveness of the renal mechanosensory nerves is modulated by dietary sodium. The ARNA and natriuretic responses to increased renal pelvic pressure are suppressed in rats fed a low-sodium diet and enhanced in rats fed a high-sodium diet (22). The modulation of the ARNA responses by dietary sodium is paralleled by the PGE2-mediated release of substance P, being suppressed in rats fed a low-sodium diet and enhanced in rats fed a high-sodium diet. Administration of the ANG type 1 (AT1) receptor antagonist losartan to the renal pelvises enhances the PGE2-mediated release of substance P and the ARNA responses to increased renal pelvic pressure in rats fed a low-sodium diet, but has no effect in rats fed a normal or high-sodium diet. Conversely, renal pelvic administration of ANG II suppresses the PGE2-mediated release of substance P and the ARNA response to increased renal pelvic pressure in rats fed a high-sodium diet (21, 22). Taken together, these studies suggest that endogenous ANG II modulates the responsiveness of the renal mechanosensory nerves by exerting an inhibitory effect on the PGE2-mediated release of substance P from the peripheral renal pelvic sensory nerve endings (22).
Suppression of the renorenal reflexes in physiological conditions of sodium retention produced by, e.g., a low-sodium diet, is an appropriate response. However, in pathological conditions of sodium retention, an impairment of the renorenal reflexes would aggravate the sodium retention. Congestive heart failure (CHF) is a condition characterized by increased ERSNA and sodium retention, the increased ERSNA contributing significantly to the sodium retention (11). There is extensive evidence for the increased ERSNA being related to an impairment of the carotid and arterial baroreceptor reflexes (11, 50). The renin-angiotensin system is activated in CHF (12, 13, 42, 44) and plays an important role in the impairment of the carotid and arterial baroreceptor reflexes (8, 10, 11).

These studies postulate a central effect of ANG II in the suppression of the baroreceptor reflexes in CHF. However, our previous studies examining the effects of physiological changes in the endogenous level of ANG II produced by alterations in dietary sodium intake (22) would indicate that ANG II, in addition to its central effect, may modulate the responsiveness of peripheral renal sensory nerves in CHF.

In the present study, we examined whether the natriuretic renorenal reflexes were impaired in CHF. We compared the responsiveness of the renal mechanosensory nerves in CHF and sham-CHF rats fed a normal sodium diet. Because these studies showed reduced responsiveness of the renal mechanosensory nerves in CHF rats, further studies were undertaken to examine the role of ANG II in the suppressed ARNA responses to increased renal pelvic pressure by studying the effects of losartan administered into the renal pelvis. In view of ANG II binding sites being located in the renal pelvic wall (15, 19, 35, 49), we also compared the PGE2-mediated release of substance P from isolated renal pelvises from CHF and sham-CHF rats treated with vehicle and losartan.

METHODS

The experimental protocols were approved by the Institutional Animal Care and Use Committee and performed according to the American Physiological Society’s “Guide for the Care and Use of Laboratory Animals.”

Male Sprague-Dawley rats allowed free access to normal sodium pellets (Teklad, sodium content 163 meq/kg) and tap water to drink were used in the study. CHF was induced by left coronary artery ligation (8–10). Briefly, the rats were anesthetized with methoxyhexital sodium (50 mg/kg ip) and an oral endotracheal tube was inserted. The rats were artificially ventilated with room air. The heart was exposed via a left thoracotomy and the left coronary artery was ligated between the pulmonary outflow tract and left atrium. The thorax was closed in layers, and negative pressure was created in the pleural cavity to inflate the lungs. Buprenorphine (0.05 mg/kg) was administered to relieve postoperative pain. After recovery from anesthesia and removal from the ventilator, the rats were returned to their cages. Sham-operated rats were exposed to similar procedures, except the left coronary artery was not ligated. The studies were performed 5–7 wk after coronary artery ligation or sham ligation.

Two experimental protocols were used in anesthetized rats and one experimental protocol in an isolated renal pelvic wall preparation. In the first protocol, we compared the ARNA and natriuretic responses to graded increases in renal pelvic pressure of 2.5–15 mmHg in CHF and sham-CHF rats. In the second protocol, we examined the effects of renal pelvic perfusion with losartan on the ARNA and natriuretic responses to increases in renal pelvic pressure of 2.5 and 7.5 mmHg in CHF and sham-CHF rats. In the third protocol, we compared the effects of PGE2 on the release of substance P from the isolated renal pelvis of CHF and sham-CHF rats incubated in a media containing losartan or losartan-vehicle.

In Vivo Studies

Anesthesia was induced with pentobarbital sodium, 0.2 mmol/kg ip, and maintained with an intravenous infusion of pentobarbital sodium, 0.04 mmol·kg⁻¹·h⁻¹ in isotonic saline at 50 μl/min into the femoral vein. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA have been previously described in detail (22–32). In short, the left kidney was approached by a flank incision, a PE-10 catheter was placed in the right ureter for collection of urine, and a PE-60 catheter was placed in the left ureter with its tip in the pelvis. The left renal pelvis was perfused, via a PE-10 catheter placed inside the PE-60 catheter, at 20 μl/min with vehicle or losartan in the second study (see below). ARNA recordings. Approximately 1.5 h elapsed after the end of surgery and the start of the experiment to allow the rat to stabilize as evidenced by 30 min of steady-state urine collections and ARNA recordings. ARNA responses to graded increases in renal pelvic pressure in CHF and sham-CHF rats. In 10 CHF rats and 10 sham-CHF rats, the left ureteral catheter was raised to increase renal pelvic pressure 2.5, 5, 7.5, 10, 12.5, and 15 mmHg. Each step increase in renal pelvic pressure was maintained for 3 min and separated by an interval of 15 min.

Effects of an AT1 receptor antagonist on the ARNA response to increased renal pelvic pressure in CHF and sham-CHF rats. Three groups were studied. The experiment was divided into two parts separated by a 10-min interval. Each part consisted of two 10-min control, 3-min experimental, and 10-min recovery periods. The left ureteral catheter was raised to increase renal pelvic pressure 2.5 and 7.5 mmHg in random order during the two experimental periods. In two groups of rats, 10 CHF rats and 10 sham-CHF rats, the renal pelvic was perfused with vehicle or losartan. In the experiment and the AT1 receptor antagonist losartan, 0.44 mM, during the second part. The renal pelvic perfusate was switched immediately after the second recovery period. In the third group of rats, eight CHF rats that served as time controls, the experimental protocol was the same, except the

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renal pelvis was perfused with vehicle throughout the experiment. At the end of each experiment, a catheter was inserted into the right carotid artery and advanced into the left ventricle for measurement of left ventricular end-diastolic pressure (LVEDP).

**In Vitro Studies**

After induction of anesthesia, as described above, LVEDP was measured in each rat before the kidneys were removed. The procedures for stimulating the release of substance P from an isolated rat renal wall preparation have been previously described in detail (20). In short, renal pelvises dissected from the kidneys were placed in wells containing 400 μl HEPES-indomethacin (0.14 μM) buffer containing various endopeptidase inhibitors maintained at 37°C. Indomethacin was included in the incubation buffer to minimize the influence of endogenous PGE2 on substance P release (20). Each well contained the pelvic wall from one kidney.

The renal pelvises were allowed to equilibrate for 130 min. The incubation medium was gently aspirated every 10 min for the first 120 min and every 5 min thereafter. The medium was immediately replaced with fresh HEPES-indomethacin buffer to maintain PO2 of the medium at 160–170 mmHg throughout the equilibration and experimental periods. In all groups, the protocol consisted of four 5-min control, one 5-min experimental, and four 5-min recovery periods. The incubation medium was placed in siliconized vials and stored at −80°C for later analysis of substance P.

**Effects of AT1 receptor blockade on the PGE2-mediated release of substance P.** One renal pelvis from each CHF rat (n = 10) and one pelvis from each sham-CHF rat (n = 10) were incubated in HEPES-indomethacin buffer (separate wells) as described above. The contralateral renal pelvises from the CHF and sham-CHF rats were incubated in HEPES-indomethacin buffer until the start of the 20-min control period when losartan, 0.44 mM, was added to the incubation buffer. Losartan was present in the incubation bath during the control, experimental, and recovery periods. During the experimental period, the ipsilateral and contralateral pelvises from each rat were exposed to PGE2, 0.14 μM. The pelvises from the CHF and sham-CHF rats were run in parallel.

**Drugs**

Losartan was supplied by Merck (Rathway, NJ). PGE2 was acquired from Cayman Chemicals (Ann Arbor, MI). All other agents were from Sigma Chemical (St. Louis, MO), unless otherwise stated. Indomethacin was dissolved together with Na2CO3 (2:1 weight ratio) in HEPES buffer. Losartan was dissolved in 0.15 M NaCl (in vivo experiments) or HEPES-indomethacin buffer (in vitro experiments).

**Analytic Procedure**

Right urinary sodium excretion measured during the experiment was expressed per gram kidney weight. Urinary sodium concentrations were determined with a flame photometer.

Substance P in the renal pelvic effluent was measured by ELISA as previously described in detail (20–23, 25, 26).

**Statistical Analysis**

Ipsilateral ARNA, systemic hemodynamics, and renal excretion were measured and averaged over each period. The effects of activation of renal mechanosensory nerves were calculated by comparing the experimental value with the average value of the bracketing control and recovery periods. The Friedman two-way analysis of variance together with the shortcut analysis of variance was used to determine differences in responses within groups and the Mann-Whitney U-test was used to determine differences in responses between groups. A significance level of 5% was chosen (45, 47). Data are expressed as means ± SE.

**RESULTS**

LVEDP averaged 20.4 mmHg in CHF rats and 4.4 mmHg in sham-CHF rats (P < 0.01, Table 1). Body weight, mean arterial pressure, and heart rate were similar in the two groups of rats.

**In Vivo Studies**

**ARNA responses to graded increases in renal pelvic pressure in CHF and sham-CHF rats.** Our previous studies showed an impaired responsiveness of renal mechanosensory nerves in conditions of sodium retention produced by a physiological intervention, such as low-sodium diet (22). CHF is a pathological condition of sodium retention (11). We therefore hypothesized that the responsiveness of the renorenal reflexes may be suppressed also in rats with CHF. Increasing renal pelvic pressure in 2.5-mmHg steps resulted in graded increases in ipsilateral ARNA (Fig. 1) and contralateral renal pelvic pressure in 2.5-mmHg steps resulted in graded increases in ipsilateral ARNA (Fig. 1) and contralateral renal mechanosensory nerve fibers was 7.5 mmHg in CHF rats vs. 5 mmHg in sham-CHF rats. The responsiveness of the renal mechanosensory nerves to increases in renal pelvic pressure of 2.5–7.5 mmHg was impaired in CHF rats. Basal ARNA was similar in CHF and sham-CHF rats, 1,350 ± 100 and 1,530 ± 100 μV·s·mmHg, respectively. Mean arterial pressure, 105 ± 1 and 107 ± 2 mmHg, and heart rate, 296 ± 11 and 297 ± 11 beats/min, did not change during the course of the experiment in the two groups of rats.

**Effects of an AT1 receptor antagonist on the ARNA response to increased renal pelvic pressure in CHF and sham-CHF rats.** In rats fed a low-sodium diet, the impairment of the natriuretic renorenal reflexes is due to increased activation of the renin-angiotensin system (22). Previous studies have shown that the current model of CHF is characterized by increased plasma
renin activity (8, 9). We tested the idea that the impaired ARNA and natriuretic responses to increases in renal pelvic pressure of 2.5 to 7.5 mmHg in CHF rats (Fig. 1) were due to increased renal ANG II levels by comparing the responses to increased renal pelvic pressure during renal pelvic perfusion with vehicle and losartan. In CHF rats during renal pelvic perfusion with vehicle, increasing renal pelvic pressure 2.6 ± 0.1 and 7.6 ± 0.1 mmHg increased ipsilateral ARNA and contralateral urinary sodium excretion (Fig. 3 and Table 2) to a similar extent as in the previous group of CHF rats (Figs. 1 and 2). Renal pelvic perfusion with losartan shifted the ARNA and natriuretic response curves to increased renal pelvic pressure up and to the left (Fig. 3 and Table 2). Thus losartan enhanced the renorenal reflex responses to increased renal pelvic pressure. Renal pelvic perfusion with losartan did not alter basal ARNA, mean arterial pressure, or heart rate, the values before and during losartan being 1,410 ± 70 and 1,320 ± 100 μV·s⁻¹, 1.12 ± 1 and 111 ± 1 mmHg, and 337 ± 4 and 333 ± 5 beats/min, respectively.

In sham-CHF rats, increasing renal pelvic pressure 2.5 ± 0 and 7.5 ± 0.1 resulted in similar ipsilateral ARNA (Fig. 3) and contralateral natriuretic responses (Table 2) during renal pelvic perfusion with vehicle and losartan. Thus losartan had no effect on the responsiveness of the renal sensory nerves in sham-CHF rats. Basal ARNA, 1,470 ± 90 μV·s⁻¹, 112 ± 1 beats/min, remained unchanged throughout the experiment.

In CHF time control experiments, increasing renal pelvic pressure 2.5 ± 0.1 and 7.9 ± 0.4 mmHg twice in...
the presence of vehicle resulted in reproducible increases in ipsilateral ARNA (Table 3). There was a small increase in contralateral urinary sodium excretion when renal pelvic pressure was increased 7.9 ± 0.4 mmHg the second time (Table 2). Mean arterial pressure and heart rate remained unaltered throughout the experiment. There was a slight decrease in basal ARNA during the course of the experiment, from 1,630 ± 80 to 1,390 ± 120 μV·s⁻¹·1 s⁻¹.

**In Vitro Studies**

**Effects of AT 1 receptor blockade on the PGE2-mediated release of substance P.** Renal pelvic perfusion with losartan enhanced the ARNA responses to increased renal pelvic pressure in CHF but not in sham-CHF rats. These data suggested that ANG II suppressed the responsiveness of the renal mechanosensory nerves in CHF (Figs. 1 and 3) by a mechanism involving the responsiveness of the renal mechanosensory nerves in rats. Furthermore, our data suggest that the mechanisms involved in the impaired responsiveness of the renal mechanosensory nerves in CHF include endogenous PGE2, which is released from pelvic tissue in CHF but not in sham-CHF rats. Furthermore, our data suggest that the mechanisms involved in the impaired responsiveness of the renal mechanosensory nerves in CHF include endogenous PGE2, which is released from pelvic tissue in CHF but not in sham-CHF rats. Adding PGE2 to the incubation bath failed to increase renal pelvic release of substance P in CHF rats (Fig. 4) but produced a reversible increase in renal pelvic release of substance P in sham-CHF rats. However, in the presence of losartan, PGE2 caused a reversible release of substance P in CHF rats that was of a similar magnitude as that produced in sham-CHF rats. Losartan had no effect on the PGE2-mediated release of substance P in sham-CHF rats, the increases in substance P release being 114 ± 20 and 120 ± 17% in absence and presence of losartan, respectively. Losartan produced similar increases in basal renal pelvic release of substance P in CHF and sham-CHF rats.

**DISCUSSION**

The results of these experiments show that the ipsilateral ARNA and contralateral natriuretic responses to increases in renal pelvic pressure within the physiological range are reduced in CHF rats compared with those in sham-CHF rats, both groups fed a normal sodium diet. Renal pelvic perfusion with losartan shifted the ipsilateral ARNA and contralateral natriuretic responses to increased renal pelvic pressure upward and to the left in CHF rats. Losartan had no effect in sham-CHF rats. Further studies in an isolated renal pelvic wall preparation from CHF and sham-CHF rats showed that PGE2 failed to increase substance P in CHF rats but caused a reversible release of substance P in sham-CHF rats. Incubating the renal pelvic tissue with losartan enhanced the PGE2-mediated release of substance P in CHF rats but not in sham-CHF rats. Taken together, these findings suggest that the renorenal reflexes are impaired in CHF rats. Furthermore, our data suggest that the mechanisms involved in the impaired responsiveness of the renal mechanosensory nerves in CHF include endoge-

### Table 2. Effects of increasing renal pelvic pressure on contralateral urinary sodium excretion in CHF and sham-CHF rats and CHF-time control rats before and during renal pelvic perfusion with vehicle and losartan

<table>
<thead>
<tr>
<th>Renal pelvic perfusion:</th>
<th>Control</th>
<th>Losartan</th>
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<tr>
<td>CHF, n = 11</td>
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<tr>
<td>Renal pelvic perfusion:</td>
<td></td>
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<tr>
<td>CHF, n = 11</td>
<td>2.6 ± 0.1</td>
<td>0.60 ± 0.22</td>
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<tr>
<td>Losartan</td>
<td>7.6 ± 0.1</td>
<td>0.89 ± 0.28</td>
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<tr>
<td>Sham-CHF, n = 10</td>
<td>2.5 ± 0</td>
<td>0.34 ± 0.06</td>
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<tr>
<td>Losartan</td>
<td>7.5 ± 0.1</td>
<td>0.40 ± 0.08</td>
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Values are means ± SE in μmol·min⁻¹·g⁻¹. CHF-time control rat was perfused with vehicle throughout the experiment. Control, average of control and recovery periods; † RPP, increased renal pelvic pressure. *P < 0.05, †P < 0.01 vs. control; ‡P < 0.05 vs. response to † RPP during vehicle.

<table>
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<tr>
<th>Renal pelvic perfusion:</th>
<th>Control</th>
<th>Losartan</th>
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<tr>
<td>CHF, n = 8</td>
<td>2.5 ± 0.1</td>
<td>0.32 ± 0.07</td>
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<tr>
<td>Losartan</td>
<td>7.9 ± 0.3</td>
<td>0.26 ± 0.06</td>
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Table 3. ARNA responses to increases in renal pelvic pressure in the presence of renal pelvic perfusion with vehicle in CHF-time control experiments

<table>
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<tr>
<th>Renal pelvic perfusion:</th>
<th>Vehicle</th>
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<tr>
<td>CHF</td>
<td>0 ± 1</td>
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<tr>
<td>Sham-CHF</td>
<td>14 ± 2*</td>
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Values are means ± SE in % of baseline afferent renal nerve activity (ARNA). *P < 0.01 vs. 0, n = 8.
nous ANG II inhibiting the PGE₂-mediated release of substance P from renal pelvic sensory nerve fibers.

**Responsiveness of Renal Mechanosensory Nerves in CHF and Sham-CHF Rats**

In agreement with our previous studies in rats fed a normal sodium diet (32), graded increases in renal pelvic pressure result in graded increases in ipsilateral ARNA with an activation threshold between 2.5 and 5 mmHg in sham-CHF rats. Likewise, graded increases in renal pelvic pressure resulted in graded increases in ARNA in CHF rats. However, in CHF rats, the ARNA response curve was shifted to the right of that in sham-CHF rats, with the activation threshold being 7.5 mmHg. Interestingly, the ARNA response curve in the CHF rats fed a normal sodium diet is similar to that in normal rats fed a low-sodium diet (22). The ARNA response curve in sham-CHF rats fed a normal sodium diet is located between the ARNA response curves in normal rats fed the high- and low-sodium diet (22). Noteworthy are the findings that the responsiveness of the renal mechanosensory nerves in CHF rats is reduced at a range that is physiologically relevant, ≤7.5 mmHg. Measurements of renal pelvic pressure in normal rats have shown that basal renal pelvic pressure varies with the volume status of the animal. In rats, renal pelvic pressure ranges from 1 to 3 mmHg during basal conditions and may reach values of 5–8 mmHg during high urine flow rate (17, 36, 46). Importantly, the results of the current study further showed that the contralateral natriuretic responses to graded increases in renal pelvic pressure parallel the ipsilateral ARNA response curves in CHF and sham-CHF rats, the natriuretic responses being impaired in CHF rats.

Acute volume expansion activates renal mechanosensory nerves resulting in an increase in ipsilateral ARNA (16, 34) and a decrease in ipsilateral ERSNA (34). CHF is characterized by increased ERSNA (11, 41). In CHF rats, the fall in ERSNA and increase in urinary sodium excretion produced by acute volume expansion are reduced (10). The impaired responses are, to a large extent, due to an impairment of the afferent and central gain of the cardiac baroreceptor reflex control of ERSNA. The impaired cardiac baroreceptor reflexes most likely also contribute to the greater positive sodium cumulative balance in CHF rats fed a high-sodium diet (10). Our current findings would suggest that the impaired renorenal reflex control of ERSNA and sodium excretion in CHF rats would contribute to the reduced natriuretic responses to acute volume expansion and to the sodium retention during a chronic sodium load in CHF.

**Mechanisms Involved in the Reduced Responsiveness of Renal Mechanosensory Nerves in CHF**

There is considerable evidence for increased activation of the renin-angiotensin system in CHF in humans (7, 12–14). Likewise, arterial plasma renin activity is increased in rats in which CHF was produced by coronary artery ligation resulting in LVEDP of a similar magnitude as in the present study, i.e., ~20 mmHg (8, 9). Previous studies have shown an important role for central ANG II in the impairment of arterial and cardiac reflex control of ERSNA (8, 9). There are few studies examining the effects of ANG II on peripheral baroreceptor activity due to difficulties in separating the effects of ANG II on afferent nerve activity per se from secondary effects related to vasoconstriction (37). However, there are reports on ANG II reducing calcium current in the nodose ganglia (3). These latter studies are of potential interest in view of our previous studies showing the PGE₂-mediated release of substance P from renal pelvic sensory nerves being dependent on calcium entry via N-type calcium channels (20).
The findings of elevated intrarenal levels of ANG II in CHF (35) suggest that ANG II may modulate the responsiveness of the renal mechanosensory nerves at the peripheral renal level in CHF rats. AT$_1$ receptors are distributed on tubular and vascular structures throughout the kidney (38, 49). In addition, AT$_1$ receptors have been demonstrated in the renal pelvic wall by autoradiography and in situ hybridization (15, 19, 35, 49). Although it is not possible to deduce from these studies whether the AT$_1$ receptors are located on the renal sensory nerve fibers in the pelvic wall, there is considerable evidence for AT$_1$ receptors on central sensory neurons (2). There are also reports of AT$_1$ receptors being associated with the peripheral aortic depressor nerve (2). We tested the idea of renal pelvic ANG II modulating the responsiveness of renal mechanosensory nerves by comparing the responses to increased renal pelvic pressure of 2.5 and 7.5 mmHg during renal pelvic perfusion with vehicle and losartan. The increases in renal pelvic pressure of 2.5 and 7.5 mmHg were chosen because they represent physiological increases in renal pelvic pressure (17, 36, 46). In CHF rats, renal pelvic perfusion with losartan shifted the ipsilateral ARNA and contralateral natriuretic response curves to increased renal pelvic pressure upward and to the left of those obtained during vehicle perfusion. Thus losartan enhanced the responsiveness of the renal mechanosensory nerves in CHF rats. Similar to our previous studies in rats fed a normal sodium diet (22), losartan had no effect on the ARNA and natriuretic responses in sham-CHF rats. Also, repeated increases in renal pelvic pressure in the presence of vehicle produced reproducible responses in CHF rats. Importantly, renal pelvic perfusion with losartan had no effect on mean arterial pressure in either group. Basal ARNA and urinary sodium excretion were also unaffected by losartan. These findings suggest that elevated levels of endogenous ANG II in CHF rats impair the responsiveness of the renal mechanosensory nerves by a peripheral mechanism resulting in an impairment of the renorenal reflex control of ERSNA and sodium excretion.

### Renal Pelvic Release of Substance P in CHF and sham-CHF Rats

Increasing renal pelvic pressure leads to a PGE$_2$-mediated release of substance P, which is a crucial mediator in the activation of renal mechanosensory nerves (22, 26, 27, 30). Our current in vivo studies indicate that ANG II reduces the responses of the renal sensory nerves in CHF by a peripheral effect on the renal sensory nerves. We used the isolated renal pelvic wall preparation to examine the hypothesis that ANG II impaired the renorenal reflexes by modulating the release of substance P. The results of the present study showed that PGE$_2$ failed to increase the release of substance P from renal pelvic tissue from CHF rats. The same concentration of PGE$_2$, 0.14 μM, resulted in a reversible increase in the release of substance P from renal pelvises from sham-CHF rats. The pelvises from CHF and sham-CHF rats were run and assayed in parallel experiments to minimize the influence of possible fluctuations in substance P release between assays. We have also repeatedly shown in previous experiments that PGE$_2$ at this concentration, 0.14 μM, results in a reversible release of substance P in rats fed a normal sodium diet (20, 22, 23). The results from our current studies further showed that incubating the renal pelvic tissue from CHF rats with losartan enhanced the PGE$_2$-mediated release of substance P to levels similar to those in vehicle- and losartan-treated renal pelvises from sham-CHF rats. Similar to our previous studies in rats fed normal sodium diet (22), losartan had no effect on the PGE$_2$-mediated release of substance P in sham-CHF rats. Taken together, these data suggest that the losartan-mediated enhancement of the ARNA response to increased renal pelvic pressure is due to losartan blocking the inhibitory effects of ANG II on the PGE$_2$-mediated release of substance P from renal pelvic sensory nerves.

Basal substance P release from pelvises from CHF and sham-CHF rats was greater in the presence than in the absence of losartan in the incubation bath. These findings are in accordance with our previous studies in rats fed a low and normal sodium diet (22). These previous studies further showed that losartan had no effect on basal substance P release from rats fed a high-sodium diet. These data would indicate that endogenous ANG II suppresses basal release of substance P from an isolated renal pelvic wall preparation. These findings would appear to be in conflict with our current and previous in vivo studies (22) that show no effect of renal pelvic administration of losartan on basal ARNA. However, it is important to note that basal ARNA and substance P release in vivo are the result of a complex interaction between reflex mechanisms and peripheral renal pelvic mechanisms, whereas in the isolated renal pelvic wall preparation the control of basal substance P release is restricted to local peripheral mechanisms. Furthermore, the lack of an effect of losartan or ANG II (22) on basal ARNA is most likely related to the renal pelvic mechanosensory nerves being minimally activated during baseline because the current experimental design involves the ureter being catheterized and renal pelvis perfused with a solution containing 0.15 M NaCl (32) using a double-lumen catheter. During these conditions, renal pelvic pressure will be close to zero and pelvic peristalsis minimal. Indirect support for a low activity of the renal pelvic mechanosensory nerves during baseline is derived from our previous studies showing no effect on basal ARNA and renal pelvic release of substance P by renal pelvic administration of COX inhibitors, powerful inhibitors of renal mechanosensory nerve activation (24, 26–28). Nevertheless, it is important to note that the isolated renal pelvic wall preparation is an excellent model to study local renal pelvic mechanisms involved in the release of substance P in response to an acute stimulation of the sensory nerves as we have shown in our previous (20, 22, 23) and current studies.
Although the source of ANG II modulating the renal sensory nerves is not known, our studies in the isolated renal pelvic wall preparation (22, current study) provide strong evidence for ANG II being present and modulated in the renal pelvic tissue. Whether ANG II and AT1 receptors are present on renal pelvic sensory nerves or in the muscle and/or uroepithelial layer surrounding the renal sensory nerves in the pelvic wall is not known. Our studies in normal rats on various dietary sodium intake suggest a powerful inhibitory role for ANG II in the activation of renal sensory nerve fibers. In conditions of low endogenous levels of ANG II, PGE2 at very low concentrations, 30 nM, results in marked increases in renal pelvic release of substance P. On the other hand, in conditions of increased activation of the renin-angiotensin system, a 125-fold higher concentration of PGE2, 3.5 μM, is required to elicit a significant, albeit reduced, increase in substance P release (22).

Other important mechanisms involved in the activation of the renal mechano sensory nerves include bradykinin, COX-2, and PGE2 (24, 26–28). Whereas plasma bradykinin levels are unaltered in patients with CHF (7), renal medullary COX-2 and renal release and urinary excretion of PGE2 are increased in various animal models of CHF (1, 6, 42). There is extensive evidence for renal COX activity and PGE2 being increased as a counterregulatory mechanism against excessive vasoconstriction and sodium reabsorption produced by increased ERSNA and/or ANG II in various models of sodium retention, including CHF (1, 4, 12, 13, 18, 39, 40, 42). Our previous studies localized COX-2 mRNA and PGE2 synthesis in the renal pelvic wall in anesthetized rats (24, 27). Assuming that changes in COX-2 activity and PGE2 synthesis in the renal pelvic wall parallel those in renal medulla, the increased renal pelvic COX-2 activity and PGE2 synthesis would serve to counteract the ANG II-mediated inhibitory effects on the natriuretic renorenal reflexes in conditions of sodium retention.

In summary, the present study shows that the ipsilateral ARNA and contralateral natriuretic responses to increasing renal pelvic pressure ≤ 7.5 mmHg are suppressed in CHF rats compared with sham-CHF rats, both groups fed a normal sodium diet. Further studies showed that the PGE2-mediated release of substance P from the renal pelvic wall is suppressed in CHF rats. Losartan enhances the ipsilateral ARNA and contralateral natriuretic responses to increased renal pelvic pressure and the PGE2-mediated substance P release in CHF rats but not in sham-CHF rats. Taken together, these findings suggest that ANG II suppresses the responsiveness of renal mechano sensory nerves in CHF rats by inhibiting the PGE2-mediated release of substance P from renal pelvic sensory nerve fibers. Suppression of the inhibitory natriuretic renorenal reflexes in CHF may contribute to the increased ERSNA and sodium retention in CHF.

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