Endogenous angiotensin modulates PGE$_2$-mediated release of substance P from renal mechanosensory nerve fibers

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Kopp, Ulla C., Michael Z. Cicha, and Lori A. Smith. Endogenous angiotensin modulates PGE$_2$-mediated release of substance P from renal mechanosensory nerve fibers. Am J Physiol Regulatory Integrative Comp Physiol 282: R19–R30, 2002.—Increasing renal pelvic pressure increases afferent renal nerve activity (ARNA) by a prostaglandin E$_2$ (PGE$_2$)-mediated release of substance P (SP) from renal pelvic sensory nerves. We examined whether the ARNA responses were modulated by high- and low-sodium diets. Increasing renal pelvic pressure resulted in greater ARNA responses in rats fed a high-sodium than in those fed a low-sodium diet. In rats fed a low-sodium diet, increasing renal pelvic pressure increased ARNA 10 ± 1 and 23 ± 3% before and 1 ± 1 and 11 ± 2% during pelvic perfusion with 15 nM ANG II. The PGE$_2$-mediated release of SP from renal pelvic nerves in vitro was enhanced in rats fed a high-sodium diet and suppressed in rats fed a low-sodium diet. The PGE$_2$ concentration required for SP release was 0.03, 0.14, and 3.5 pg/min with losartan in the incubation bath. Losartan had no effect on SP release in rats fed normal- and high-sodium diets. ANG II modulates the responsiveness of renal pelvic mechanosensory nerves by inhibiting PGE$_2$-mediated SP release from renal pelvic nerve fibers.

AT$_1$ receptors; losartan; kidney; high-sodium diet; low-sodium diet; afferent renal nerves

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ies showed that a high-sodium diet enhanced and a low-sodium diet suppressed the ARNA responses to activation of the renal pelvic mechanosensory nerves, we hypothesized that ANG II may play a role in the altered responsiveness of the renal mechanosensory nerve fibers. Interestingly, angiotensin type 1 (AT1) receptors have been localized in the renal pelvic wall by in situ hybridization and autoradiography (14, 18, 37, 53). Therefore, we examined whether renal pelvic perfusion with an AT1 receptor antagonist, losartan, and ANG II altered the ARNA responses to increased renal pelvic pressure in rats fed low- and high-sodium diets, respectively. The results from these studies suggested that endogenous ANG II modulates the responsiveness of renal mechanosensory nerves by an effect on sensory nerves in the renal pelvic wall.

AT1 receptors are widely distributed in areas in the central nervous system associated with cardiovascular control, including sensory neurons in the dorsal root ganglia and nodose ganglia (2, 42). Interestingly, ANG II binding sites have been shown to be transported peripherally in the aortic depressor nerve in the rat (2). In the nucleus tractus solitarius, AT1 receptors are present on substance P-containing vagal afferent nerve fibers, and ANG II has been shown to increase the release of substance P (3). Although ANG II is generally considered to be an excitatory neurotransmitter (2), there are reports that ANG II inhibits calcium current through N-type calcium channels in the sensory nodose ganglion (4). Because these studies suggested that ANG II may modulate the release of substance P from sensory nerve fibers, we examined whether changes in dietary sodium intake modulate the PGE2-mediated release of substance P from an isolated renal pelvic wall preparation.

METHODS

The study was performed on male Sprague-Dawley rats weighing 220–410 g (mean 307 ± 3 g). Two weeks before the study, rats were placed on sodium-deficient pellets (ICN, sodium = 1.6 meq/kg) with tap water drinking fluid (low-sodium diet, n = 64), normal-sodium pellets (Teklad, sodium = 163 meq/kg) with tap water drinking fluid (normal-sodium diet, n = 54), or normal-sodium pellets with 0.9% NaCl drinking fluid (high-sodium diet, n = 52) (9, 11).

In Vivo Studies

Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg ip) and maintained with an infusion of pentobarbital sodium (0.04 mmol·kg⁻¹·h⁻¹ iv) 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 (22–30). Briefly, 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, throughout the experiment at 20 μL/min with vehicle or various renal perfusates administered in the different experimental protocols. ARNA was stimulated by increasing renal pelvic pressure. Renal pelvic pressure was increased by elevating the fluid-filled catheter above the level of the kidney. ARNA was recorded from the peripheral portion of the cut end of one renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-s intervals and measured in microvolts per second per 1 s. Postmortem renal nerve activity, which was assessed by crushing the decentralized renal nerve bundle peripheral to the recording electrode, was subtracted from all values of renal nerve activity. ARNA was expressed as a percentage of its baseline value during the control period (22–30).

Experimental Protocols

Approximately 1.5 h elapsed between 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.

Effects of dietary sodium on ARNA responses to graded increases in renal pelvic pressure. In rats fed the low-sodium (n = 11) and high-sodium (n = 10) diets, the left ureteral catheter was raised to increase renal pelvic pressure 2.5, 5, 7.5, 10, 12.5, and 15 mmHg at 15-min intervals. Each step increase in renal pelvic pressure was maintained for 3 min. The renal pelvis was not perfused.

Effects of an AT1 receptor antagonist on the ARNA response to increased renal pelvic pressure in rats fed the low- and normal-sodium diets. Three groups were studied: two groups were fed the low-sodium diet, and one group was fed the normal-sodium diet. 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, one group fed the low-sodium diet (n = 8) and one group fed the normal-sodium diet (n = 9), the renal pelvis was perfused with vehicle during the first part of 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, which was fed the low-sodium diet (n = 10) and served as time controls, the experimental protocol was the same, except the renal pelvis was perfused with vehicle throughout the experiment.

Effects of ANG II on ARNA responses to increased renal pelvic pressure in rats fed the high-sodium diet. The left ureteral catheter was raised to increase renal pelvic pressure 2.5 and 7.5 mmHg using an experimental protocol similar to that described above for the groups fed the low- and normal-sodium diets. Two groups fed the high-sodium diet were studied; in the first group (n = 8), the renal pelvis was perfused with vehicle during the first part of the experiment and 15 nM ANG II during the second part, and in the second group (n = 11), which served as time controls, the experimental protocol was the same, except the renal pelvis was perfused with vehicle throughout the experiment.

In Vitro Studies

The procedures for stimulating the release of substance P from an isolated rat renal pelvic wall preparation have been previously described in detail (21). Briefly, renal pelvises dissected from the kidneys were placed in wells containing 400 μL of 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 (21). Each well contained the pelvic wall from one kidney.

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The renal pelvic walls 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 P02 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 dietary sodium on PGE2-mediated release of substance P. Two groups were studied. In the first group, renal pelvises from rats fed the low- and normal-sodium diets were exposed to 0.14, 0.70, or 3.5 μM PGE2 during the experimental period, the ipsilateral and contralateral pelvises being exposed to different concentrations of PGE2. The pelvises from rats fed the low- and normal-sodium diets were run in parallel. In the second group, consisting of renal pelvises from rats fed the high- and normal-sodium diets, the experimental protocol was the same, except these pelvises were exposed to 0.03, 0.14, or 0.70 μM PGE2 during the experimental period.

Effects of AT1 receptor blockade on PGE2-mediated release of substance P. Two groups were studied. In the first group, one renal pelvis from rats fed the low- and normal-sodium diets was incubated in HEPES-indomethacin buffer as described above. The contralateral renal pelvis was incubated in HEPES-indomethacin buffer until the start of the 20-min control period, when 0.44 mM losartan was added to the incubation buffer. During the experimental period, the ipsilateral and contralateral pelvises from each rat was exposed to the same concentration of PGE2, 0.14, 0.70, or 3.5 μM. The pelvises from rats fed the low- and normal-sodium diets were run in parallel. In the second group, consisting of renal pelvises from rats fed the high-sodium diet, the experimental protocol was the same, except these pelvises were exposed to 0.03 μM PGE2.

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

Analytic Procedure
After the rats were fed the low- or high-sodium diet for 2 wk, they were placed in metabolic cages for 48 h for measurements of urinary sodium excretion. Right urinary sodium excretion measured during the experiment was expressed per gram of 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 (21, 24).

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 were used to determine differences in responses within groups and the Mann-Whitney U test to determine differences in responses between groups. A significance level of 5% was chosen (43, 46). Values are means ± SE.

RESULTS
In rats fed the low- and high-sodium diets for ≥2 wk, urinary sodium excretion averaged 160 ±20 and 7,040 ±490 μmol/24 h, respectively, in the conscious state.

In Vivo Studies
Effects of dietary sodium on ARNA responses to graded increases in renal pelvic pressure. The natriuretic nature of the renorenal reflexes (23–30) and the regulation of various renal mediators involved in the activation of the afferent renal nerves by dietary sodium intake (17, 31, 51) suggest that changes in dietary sodium may influence the responsiveness of renal mechanosensory nerve terminals. We tested this hypothesis by comparing the responses of ipsilateral ARNA and contralateral urinary sodium excretion to graded increases in renal pelvic pressure in rats fed the high- and low-sodium diets. Increasing renal pelvic pressure in 2.5-mmHg steps resulted in graded increases in ipsilateral ARNA (Fig. 1) and contralateral urinary sodium excretion (Fig. 2) in rats fed the high- or low-sodium diet. However, the curves depicting the responses of ARNA and urinary sodium excretion in rats fed the high-sodium diet were to the left of those in rats fed the low-sodium diet. Thus each increase in renal pelvic pressure resulted in a greater ARNA and
natriuretic response in rats fed the high-sodium diet than in rats fed the low-sodium diet. The threshold for activation of the renal mechanosensory nerve fibers was 2.6 mmHg in rats fed the high-sodium diet and 7.6 mmHg in rats fed the low-sodium diet. Mean arterial pressure (114 ± 0.11006 and 111 ± 0.13006 mmHg) and heart rate (350 ± 25 and 323 ± 18 beats/min), similar in rats fed the high- and low-sodium diets, did not change during the course of the experiment.

Effects of an AT1 receptor antagonist on the ARNA response to increased renal pelvic pressure in rats fed the low- and normal-sodium diets. Variation in sodium intake produces physiological changes in endogenous ANG II (9, 11). ERNA and its baroreflex control are modulated by ANG II of central nervous system origin (9, 11). However, the demonstration of AT1 receptors in the muscle wall of the renal pelvis (37), where the majority of the renal sensory nerve fibers are located (24), led us to hypothesize that the impaired ARNA responses to increased renal pelvic pressure in rats fed the low-sodium diet were related to an effect of ANG II on the mechanosensitive nerve terminals in the renal pelvic wall. We tested this idea by examining whether renal pelvic perfusion with losartan would alter the ARNA responses to increased renal pelvic pressure in rats fed the low-sodium diet. During renal pelvic perfusion with vehicle, increasing renal pelvic pressure 2.8 ± 0.1 and 7.7 ± 0.1 mmHg resulted in similar ARNA responses (Fig. 3) similar to those observed in the previous group of rats fed the low-sodium diet (Fig. 1). Renal pelvic perfusion with losartan shifted the ARNA response curve to increased renal pelvic pressure upward and to the left. Thus losartan enhanced the ARNA responses to increased renal pelvic pressure. The contralateral natriuretic responses to increased renal pelvic pressure paralleled the ARNA responses (Table 1). Renal pelvic perfusion with losartan did not alter basal ARNA, mean arterial pressure, or heart rate: 1,486 ± 131 and 1,461 ± 124 μV·s·s⁻¹, 105 ± 4 and 105 ± 4 mmHg, and 374 ± 28 and 369 ± 30 beats/min before and during perfusion with losartan, respectively.

In the time control experiments in rats fed the low-sodium diet, increasing renal pelvic pressure 2.7 ± 0.1 and 7.5 ± 0.1 mmHg in the presence of vehicle resulted in reproducible increases in ipsilateral ARNA (Fig. 3B) and contralateral urinary sodium excretion (Table 1) that were similar in magnitude to those in the previous group during vehicle administration (Fig. 3A). Basal ARNA (1,626 ± 102 μV·s·s⁻¹), mean arterial pressure...
renal pelvic perfusion with vehicle and losartan. Basal contralateral urinary sodium excretion (Table 1) during similar increases in ipsilateral ARNA (Fig. 4) and renal pelvic pressure 2.6 mmHg remained unchanged throughout the experiment.

Fig. 4. Effects of increasing renal pelvic pressure 2.6 mmHg during renal pelvic perfusion with vehicle and losartan on ARNA (paired experimental design) in rats fed the normal-sodium diet. **P < 0.01 vs. zero.

In rats fed the normal-sodium diet, increasing renal pelvic pressure 2.6 ± 0.1 and 7.6 ± 0.1 mmHg resulted in similar increases in ipsilateral ARNA (Fig. 4) and contralateral urinary sodium excretion (Table 1) during renal pelvic perfusion with vehicle and losartan. Basal ARNA (1.582 ± 87 μV·s·s⁻¹) and mean arterial pressure (112 ± 2 mmHg), and heart rate (354 ± 11 beats/min) remained unchanged throughout the experiment.

**Effects of ANG II on ARNA responses to increased renal pelvic pressure in rats fed the high-sodium diet.** Our studies showing that renal pelvic perfusion with losartan enhanced the responsiveness of the renal mechanosensory nerve fibers in rats fed the low-sodium diet suggested that the renin-angiotensin system exerted an inhibitory effect on the activation of the renal pelvic sensory nerve fibers. We tested this idea by examining the effects of renal pelvic perfusion with ANG II on the ARNA responses to increased renal pelvic pressure in rats fed the high-sodium diet. In the presence of vehicle, increasing renal pelvic pressure 2.7 ± 0.1 and 7.9 ± 0.1 mmHg resulted in similar increases in ipsilateral ARNA (Fig. 5) as observed in the previous group of rats fed the high-sodium diet (Fig. 1). Renal pelvic perfusion with ANG II shifted the ARNA response curve downward and to the right. Thus ANG II suppressed the ARNA responses to increased renal pelvic pressure. Increasing renal pelvic pressure 2.7 ± 0.1 and 7.9 ± 0.1 mmHg increased contralateral urinary sodium excretion 12 ± 8% from 1.4 ± 0.2 μmol·min⁻¹·g⁻¹ and 63 ± 25% (P < 0.01) from 1.4 ± 0.3 μmol·min⁻¹·g⁻¹, respectively, during vehicle and 9 ± 3% from 2.1 ± 0.4 μmol·min⁻¹·g⁻¹ and 36 ± 10% (P < 0.02) from 2.0 ± 0.3 μmol·min⁻¹·g⁻¹, respectively, during ANG II. Renal pelvic perfusion with ANG II did not alter basal ARNA, mean arterial pressure, or heart rate: 1,457 ± 145 and 1,445 ± 176 μV·s·s⁻¹, 107 ± 3 and 107 ± 2 mmHg, and 308 ± 23 and 309 ± 24 beats/min before and during perfusion with ANG II, respectively.

In time control experiments, increasing renal pelvic pressure 2.6 ± 0 and 7.8 ± 0.1 mmHg resulted in reproducible increases in ipsilateral ARNA (Fig. 5B) that were similar in magnitude to those in the previous group of rats fed the high-sodium diet during vehicle perfusion (Fig. 5A). Increasing renal pelvic pressure 2.6 ± 0 and 7.8 ± 0.1 mmHg increased contralateral urinary sodium excretion 33 ± 7% from 1.0 ± 0.2 μmol·min⁻¹·g⁻¹ and 41 ± 17% from 1.1 ± 0.2 μmol·min⁻¹·g⁻¹, respectively, during vehicle I and

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Values are means ± SE in μmol·min⁻¹·g⁻¹. Control, average of control and recovery periods; ↑ RPP, increased renal pelvic pressure. *P < 0.05, †P < 0.01 vs. control.

(107 ± 3 mmHg), and heart rate (297 ± 12 beats/min) remained unchanged throughout the experiment.

The pressure increased contralateral ARNA (Fig. 4) and renal pelvic pressure 2.6 mmHg remained unchanged throughout the experiment. In rats fed the normal-sodium diet, increasing renal pelvic pressure 2.6 ± 0.1 and 7.6 ± 0.1 mmHg resulted in similar increases in ipsilateral ARNA (Fig. 4) and contralateral urinary sodium excretion (Table 1) during renal pelvic perfusion with vehicle and losartan. Basal ARNA (1.582 ± 87 μV·s·s⁻¹), mean arterial pressure (112 ± 2 mmHg), and heart rate (354 ± 11 beats/min) remained unchanged throughout the experiment. ANG II on the ARNA responses to increased renal pelvic pressure in rats fed the high-sodium diet using the paired experimental design in rats fed the normal-sodium diet. Our studies showing that renal pelvic perfusion with losartan enhanced the responsiveness of the renal mechanosensory nerve fibers in rats fed the low-sodium diet suggested that the renin-angiotensin system affected the mechanism of the activation of the renal pelvic sensory nerve fibers. We tested this idea by examining the effects of renal pelvic perfusion with ANG II on the ARNA responses to increased renal pelvic pressure in rats fed the high-sodium diet. In the presence of vehicle, increasing renal pelvic pressure 2.7 ± 0.1 and 7.9 ± 0.1 mmHg resulted in similar increases in ipsilateral ARNA (Fig. 5) as observed in the previous group of rats fed the high-sodium diet (Fig. 1). Renal pelvic perfusion with ANG II shifted the ARNA response curve downward and to the right. Thus ANG II suppressed the ARNA responses to increased renal pelvic pressure. Increasing renal pelvic pressure 2.7 ± 0.1 and 7.9 ± 0.1 mmHg increased contralateral urinary sodium excretion 12 ± 8% from 1.4 ± 0.2 μmol·min⁻¹·g⁻¹ and 63 ± 25% (P < 0.01) from 1.4 ± 0.3 μmol·min⁻¹·g⁻¹, respectively, during vehicle and 9 ± 3% from 2.1 ± 0.4 μmol·min⁻¹·g⁻¹ and 36 ± 10% (P < 0.02) from 2.0 ± 0.3 μmol·min⁻¹·g⁻¹, respectively, during ANG II. Renal pelvic perfusion with ANG II did not alter basal ARNA, mean arterial pressure, or heart rate: 1,457 ± 145 and 1,445 ± 176 μV·s·s⁻¹, 107 ± 3 and 107 ± 2 mmHg, and 308 ± 23 and 309 ± 24 beats/min before and during perfusion with ANG II, respectively.

In time control experiments, increasing renal pelvic pressure 2.6 ± 0 and 7.8 ± 0.1 mmHg resulted in reproducible increases in ipsilateral ARNA (Fig. 5B) that were similar in magnitude to those in the previous group of rats fed the high-sodium diet during vehicle perfusion (Fig. 5A). Increasing renal pelvic pressure 2.6 ± 0 and 7.8 ± 0.1 mmHg increased contralateral urinary sodium excretion 33 ± 7% from 1.0 ± 0.2 μmol·min⁻¹·g⁻¹ and 41 ± 17% from 1.1 ± 0.2 μmol·min⁻¹·g⁻¹, respectively, during vehicle I and
36 ± 9% from 1.6 ± 0.4 μmol·min⁻¹·g⁻¹ and 37 ± 9% from 1.4 ± 0.3 μmol·min⁻¹·g⁻¹, respectively, during vehicle II. Basal ARNA (1,676 ± 97 μV·s·s⁻¹), mean arterial pressure (114 ± 2 mmHg), and heart rate (303 ± 12 beats/min) remained unchanged throughout the experiment.

In Vitro Studies

Effects of dietary sodium on PGE₂-mediated release of substance P. The increase in ARNA produced by increased renal pelvic pressure is due to a PGE₂-dependent release of substance P from sensory nerves in the renal pelvic wall (21). Our in vivo findings showing that renal pelvic perfusion with losartan enhanced and ANG II suppressed the ARNA responses to increased renal pelvic pressure in rats fed the low- and high-sodium diets, respectively, suggest that endogenous ANG II alters the responsiveness of the renal mechanosensory nerve fibers by modulating the release of substance P. We tested this idea in the isolated renal pelvic wall preparation by examining whether dietary sodium affected the PGE₂-mediated release of substance P.

Fig. 5. A: effects of increasing renal pelvic pressure 2.7 ± 0.1 and 7.9 ± 0.1 mmHg during renal pelvic perfusion with vehicle and 15 nM ANG II on ARNA (paired experimental design) in rats fed the high-sodium diet. B: effects of increasing renal pelvic pressure 2.6 ± 0 and 7.8 ± 0.1 mmHg during renal pelvic perfusion with vehicle on ARNA (time control experiments) in rats fed the high-sodium diet. **P < 0.01 vs. zero. †P < 0.05; ‡P < 0.01 vs. ARNA response during vehicle.

In rats fed the normal-sodium diet, 0.14, 0.7, and 3.5 μM PGE₂ resulted in reversible increases in the renal pelvic release of substance P (P < 0.01; Fig. 6, Table 2). In rats fed the low-sodium diet, 0.14 and 0.7 μM PGE₂ failed to increase renal pelvic release of substance P (Fig. 6, Table 2). PGE₂ at 3.5 μM produced a small increase in renal pelvic release of substance P that was

Fig. 6. Effects of 0.14 μM PGE₂ on release of substance P from an isolated renal pelvic wall preparation from rats fed normal- and low-sodium diets. **P < 0.01 vs. baseline substance P release during control and recovery periods. †P < 0.01, PGE₂-mediated substance P release in rats fed the low-sodium diet vs. those fed the normal-sodium diet.
PGE$_2$ at 0.03 μM produced a reversible increase in the renal pelvic release of substance P in rats fed the high-sodium diet (P < 0.01) but not in rats fed the normal-sodium diet (Fig. 7). PGE$_2$ at 0.14 and 0.7 μM increased renal pelvic release of substance P in rats fed the normal- and high-sodium diets (Table 3). The increase in substance P produced by 0.7 μM PGE$_2$ was significantly greater in rats fed the high-sodium diet than in rats fed the normal-sodium diet (P < 0.01).

**Effects of AT$_1$ receptor blockade on the PGE$_2$-mediated release of substance P.** In rats fed the low-sodium diet, 0.14 and 0.7 μM PGE$_2$ failed to increase renal pelvic release of substance P in the absence of losartan in the incubation media (Fig. 8, Table 4), i.e., results similar to those observed in the previous groups of rats fed the low-sodium diet (Fig. 6, Table 2). However, when losartan was present in the incubation bath, 0.14 and 0.7 μM PGE$_2$ produced reversible marked increases in the release of substance P (P < 0.01), which were significantly greater (P < 0.02) than those produced in the absence of losartan (Fig. 8, Table 4). Likewise, the increase in renal pelvic release of substance P produced by 3.5 μM PGE$_2$ was significantly greater (P < 0.02) in the presence than in the absence of losartan in rats fed the low-sodium diet: 11.0 ± 1.4 (P < 0.01) and 3.5 ± 1.2 pg/min (P < 0.02), respectively. Basal substance P release was significantly increased (P < 0.01) in the presence of losartan in all groups.

In rats fed the normal-sodium diet, 0.14 and 0.7 μM PGE$_2$ produced similar increases in renal pelvic release of substance P in the absence and presence of losartan in the incubation bath (Fig. 8, Table 4). PGE$_2$ at 3.5 μM produced a slightly greater increase in renal pelvic release in the presence than in the absence of losartan: 18.4 ± 2.8 vs. 11.0 ± 2.5 pg/min (both P < 0.01). In the absence of losartan, the increases in substance P release produced by 0.14, 0.7, and 3.5 μM PGE$_2$ were greater in rats fed the normal-sodium diet than in rats fed the low-sodium diet, i.e., results similar to those observed in the previous groups (Fig. 6, Table 2).

**DISCUSSION**

The results of these experiments show that the ipsilateral ARNA and contralateral natriuretic responses to increased renal pelvic pressure are greater in rats fed the normal-sodium diet than in rats fed the low-sodium diet.

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**Table 2. Effects of dietary sodium on PGE$_2$-mediated release of substance P in an isolated renal pelvic wall preparation**

<table>
<thead>
<tr>
<th>Diet</th>
<th>PGE$_2$ (0.70 μM) n</th>
<th>Control</th>
<th>PGE$_2$ (3.5 μM) n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NaCl</td>
<td>9.0 ± 1.3</td>
<td>20.7 ± 2.9‡</td>
<td>5.5 ± 1.1</td>
</tr>
<tr>
<td>Low NaCl</td>
<td>7.6 ± 1.3</td>
<td>9.9 ± 2.0§</td>
<td>5.4 ± 1.3</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/min. PGE$_2$, prostaglandin E$_2$; control, average of control and recovery periods. *P < 0.05; †P < 0.01 vs. control. §P < 0.01 vs. normal-NaCl diet.

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**Table 3. Effects of dietary sodium on PGE$_2$-mediated release of substance P in an isolated renal pelvic wall preparation**

<table>
<thead>
<tr>
<th>Diet</th>
<th>PGE$_2$ (0.14 μM) n</th>
<th>Control</th>
<th>PGE$_2$ (0.70 μM) n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NaCl</td>
<td>3.9 ± 0.5</td>
<td>9.9 ± 1.9*</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>High NaCl</td>
<td>5.6 ± 1.6</td>
<td>13.5 ± 3.2*</td>
<td>7.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/min. Control, average of control and recovery periods. *P < 0.01 vs. control. †P < 0.01 vs. normal NaCl diet.
an enhanced release of substance P from renal pelvic sensory nerve fibers.

**Effects of Dietary Sodium on the Responsiveness of Renal Mechanosensory Nerves**

In rats fed a normal-sodium diet, graded increases in renal pelvic pressure result in graded increases in ARNA with activation threshold of 2.5–5 mmHg (30). Likewise, graded increases in renal pelvic pressure

Table 4. Effects of losartan on PGE$_2$-mediated release of substance P in rats fed normal- and low-sodium diets in an isolated renal pelvic wall preparation

<table>
<thead>
<tr>
<th>Diet</th>
<th>Vehicle Control PGE$_2$ (0.70 μM)</th>
<th>Vehicle Control PGE$_2$ (0.70 μM)</th>
<th>Losartan Control PGE$_2$ (0.70 μM)</th>
<th>Losartan Control PGE$_2$ (0.70 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal NaCl</td>
<td>8 6.4±1.2</td>
<td>22.1±5.5</td>
<td>9.9±1.4</td>
<td>25.4±4.0</td>
</tr>
<tr>
<td>Low NaCl</td>
<td>8 7.7±1.5</td>
<td>8.4±1.9</td>
<td>12.1±1.5</td>
<td>20.6±3.1</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/min. Losartan was administered at 0.44 mM and PGE$_2$ at 0.70 μM. Control, average of control and recovery periods. *P < 0.01 vs. control. †P < 0.01 vs. vehicle.
resulted in graded increases in ARNA in rats fed high-
and low-sodium diets. However, the ARNA response
curve in rats fed the high-sodium diet is shifted to the
left of that in rats fed the low-sodium diet, with the
threshold for activation of the renal pelvic mech-
anosensory nerve fibers being ≤2.6 and 7.6 mmHg in
rats fed the high- and low-sodium diets, respectively.
The parallel shift in the ARNA response curves pro-
duced by dietary sodium suggests that changes in
dietary sodium modulate the responsiveness of renal
pelvic mechanosensory nerve fibers. Importantly, the
functional responses to increased renal pelvic pres-
sure, i.e., the contralateral natriuretic responses, par-
alleled the changes in ipsilateral ARNA. Thus each
step increase in renal pelvic pressure resulted in a
greater increase in contralateral urinary sodium excre-
tion in rats fed the high-sodium diet than in those fed
the low-sodium diet. Noteworthy are our findings that
increases of renal pelvic pressure of as little as 2.6
mmHg resulted in significant increases in contralat-
eral urinary sodium excretion in rats fed the high-
sodium diet. Measurements of renal pelvic pressure in
rats would suggest that the afferent renal nerves are
activated by physiological increases in pelvic pressure.

Mechanisms Involved in the Altered Sensitivity of the
Renorenal Reflexes Produced by Dietary Sodium

In analogy with our findings are the numerous stud-
ies showing that a high-sodium diet enhances and a
low-sodium diet suppresses the sensitivity of the arte-
rial baroreflex control of ERNA in normotensive rats
(19, 41). The enhanced sensitivity of the arterial
baroreflexes produced by a high-sodium diet is, at least
in part, due to a central mechanism (41). Variation in
sodium intake produces physiological changes in en-
dogenous ANG II. Previous studies have shown that
similar low and high dietary sodium regimens as used
in the present study result in activation and suppres-
sion of the renin-angiotensin system, respectively (9,
11). It is well established that the brain renin-angio-
tensin system tonically influences arterial pressure
and baroreceptor regulation of ERNA (9, 11). Admin-
istering an AT1 receptor antagonist into the lateral
cerebral ventricle or rostral ventrolateral medulla
shifted the baroreflex curve of ERNA to changes in
mean arterial pressure toward a lower level of mean
arterial pressure without changing the gain (9, 11).
The effect was most pronounced in rats fed the low-
sodium diet, suggesting that central ANG II contrib-
utes to the increased ERNA in rats fed the low-sodium
diet.

AT1 receptors are widely distributed in the central
and peripheral nervous system, including sensory neu-
rons in the dorsal root ganglion and nodose ganglia (2).
In the kidney, AT1 receptors are located on vascular
tissues showing that a high-sodium diet enhances and a
normal-sodium diet. Conversely, in rats fed the high-
sodium diet, renal pelvic perfusion with ANG II shifted
the ARNA response curve to increased renal pelvic pressure upward and to the left in rats fed the low-sodium diet. In the presence of losartan, the threshold for activation of the renal mech-
anosensory nerves was ≥2.8 mmHg, i.e., similar to the
activation threshold observed in rats fed the high-
sodium diet. Losartan enhanced the contralateral na-
triuretic response to increasing renal pelvic pressure
2.8 mmHg, but not 7.7 mmHg. The imperfect relation-
ship between ipsilateral ARNA and contralateral uri-
inary sodium excretion during graded increases in renal
pelvic pressure may be related to the design of the
experimental protocol. Our present and previous stud-
ies (30) showed that graded increases in renal pelvic pressure resulted in graded increases in ARNA when
each step of renal pelvic pressure was held for 3 min
and the steps were separated by 10- to 15-min inter-
vals, during which renal pelvic pressure was allowed to
return to baseline. However, it is likely that the periods
during which renal pelvic pressure was increased and
then allowed to return to baseline were of insufficient
duration to allow contralateral urinary sodium excre-
tion to achieve new steady-state values. The contralat-
eral responses to activation of renal mechanosensory
nerves are the results of a complex series of events
including central integrations of the renorenal reflexes
and the intrarenal neural mechanisms in the con-
tralateral kidney (12).

Importantly, renal pelvic perfusion with losartan
had no effect on the ARNA responses to increased renal
pelvic pressure of similar magnitudes in rats fed the
normal-sodium diet. Conversely, in rats fed the high-
sodium diet, renal pelvic perfusion with ANG II shifted
the ARNA response curve to increased renal pelvic
pressure downward and to the right. Interestingly, the
ARNA response curves in the presence of losartan in
rats fed the low-sodium diet and vehicle in rats fed the
high-sodium diet were almost superimposable, as were the ARNA curves in the presence of vehicle in rats fed the low-sodium diet and ANG II in rats fed the high-sodium diet. The parallel shifts of the ARNA response curves by losartan and ANG II in the rats fed the low- and high-sodium diets, respectively, suggest that the responsiveness of the renal mechanosensory nerve fibers is modulated by endogenous ANG II. It is not likely that the effects of dietary sodium on the activation of renal mechanosensory nerves are due to altered expression of AT1 receptors on renal sensory nerve terminals, since the ARNA responses to increased renal pelvic pressure were enhanced and suppressed by acute renal pelvic perfusion with losartan and ANG II, respectively, in rats fed the low- and high-sodium diets. Importantly, renal pelvic perfusion with losartan and ANG II did not affect mean arterial pressure or heart rate. Taken together, these studies suggest that ANG II alters the responsiveness of renal pelvic mechanosensory nerves by a peripheral renal mechanism. These findings appear to be in contrast to the extensive evidence for an almost exclusive central role for ANG II modulating the baroreflexes (2, 3, 9, 11, 42). However, there are few studies examining whether ANG II may also alter sympathetic nerve activity via a direct effect on peripheral carotid and/or aortic baroreceptors. In the study by Munch and Longhurst (39), the inhibitory effect of ANG II on aortic baroreceptor activity could not be differentiated from the concomitant vasoconstriction produced by ANG II. On the other hand, the presence of ANG II binding sites on the aortic depressor nerve (2) suggests that ANG II may have the capability of modulating the activity of the peripheral sensory nerves involved in the baroreflexes.

Role of Angiotensin on the PGE2-Mediated Release of Substance P From Renal Pelvic Sensory Nerves

To test our hypothesis that ANG II exerts its inhibitory effect on the ARNA responses to increased renal pelvic pressure by an effect on renal pelvic sensory nerve fibers, we compared the PGE2-mediated release of substance P in an isolated renal pelvic wall preparation from rats fed various levels of dietary sodium. Our results showed that the PGE2-mediated release of substance P from the renal pelvic nerves was enhanced in rats fed the high-sodium diet and suppressed in rats fed the low-sodium diet compared with rats fed the normal-sodium diet. In pelvises from rats fed the high-sodium diet, the threshold concentration of PGE2 for release of substance P was 0.03 μM vs. 0.14 μM in pelvises from rats fed the normal-sodium diet. [PGE2 at 6 nM failed to increase substance P release in rats fed the high-sodium diet, from 6.8 ± 1.3 to 7.9 ± 1.8 pg/min (n = 4).] In pelvises from rats fed the low-sodium diet, 3.5 μM PGE2 was required to produce a significant, albeit suppressed, increase in the release of substance P. Thus the shifts in the ARNA response curves to increased renal pelvic pressure, to the left in rats fed the high-sodium diet and to the right in rats fed the low-sodium diet, are paralleled by shifts in the threshold concentrations of PGE2 for substance P release.

The presence of AT1 receptors in the muscle layer of the renal pelvic wall (37), where the majority of the renal sensory nerve fibers (13, 24, 33, 53) are located, suggested that the effects produced by dietary sodium on PGE2-mediated renal pelvic release of substance P were due to changes in endogenous ANG II levels in the renal pelvic wall. This hypothesis was tested by incubating pelvises from rats fed low-, normal-, and high-sodium diets with losartan. In rats fed the low-sodium diet, losartan enhanced the release of substance P produced by ≥0.14 μM PGE2 to levels similar to those in vehicle-treated pelvises from rats fed the normal-sodium diet. Importantly, losartan had no effect on PGE2-mediated substance P release in pelvises from rats fed the normal- and high-sodium diets. These studies suggest that ANG II exerts an inhibitory effect on PGE2-mediated substance P release from renal pelvic sensory nerve terminals. Furthermore, our in vitro studies suggest that the losartan-mediated enhancement of the ARNA responses to increased renal pelvic pressure in vivo is due to losartan blocking the inhibitory effects of ANG II on the PGE2-mediated release of substance P.

The inhibitory role of ANG II on the PGE2-mediated release of substance P from the isolated renal pelvic wall raises the question of the source of ANG II. There is evidence for neurons containing ANG II in areas in the central nervous system associated with cardiovascular control (42, 45). Whether ANG II is present in the renal pelvic sensory nerves, the muscle layer, and/or uroepithelium surrounding the sensory nerves in the renal pelvic wall is not known. If it is assumed that ANG II levels in the renal pelvic wall parallel intrarenal ANG II levels, it is likely that changes in dietary sodium modulate renal pelvic ANG II levels (40). It is rather unlikely that the responsiveness of the renal pelvic sensory nerves in vivo would be modified by

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**Fig. 10.** Increasing renal pelvic pressure increases release of bradykinin, which activates protein kinase C (PKC), which in turn leads to activation of cyclooxygenase-2 (COX-2) and increased PGE2 synthesis. PGE2 elicits a renal pelvic release of substance P with a resultant increase in ARNA (21, 23–29). ANG II exerts an inhibitory influence (dotted line) on PGE2-mediated release of substance P from renal pelvic sensory nerve fibers.
urinary ANG II, since changes in dietary sodium do not alter urinary ANG II levels (48).

The mechanisms involved in the inhibitory effect of ANG II on substance P release are not known. However, studies in nodose ganglia showing that ANG II results in a reduction of calcium current by blocking N-type calcium channels (4) are of potential interest considering that the PGE_2-mediated release of substance P from the renal pelvic nerves is blocked by an N-type calcium channel blocker (21).

The present studies focused on the mechanisms distal to PGE_2 synthesis that are influenced by dietary sodium intake (Fig. 10). However, it is important to note that alterations in dietary sodium intake most likely also affect renal PGE_2 synthesis per se (17, 32, 52), which could act in concert with changes in the activity of the renin-angiotensin system to modulate the release of substance P.

In summary, the present study shows that the ARNA responses to increased renal pelvic pressure in vivo and the PGE_2-mediated release of substance P in vitro are enhanced by the high-sodium diet and suppressed by the low-sodium diet. In rats fed the low-sodium diet, losartan enhances the ARNA responses to increased renal pelvic pressure and the PGE_2-mediated release of substance P to levels similar to those in vehicle-treated rats fed the normal- and high-sodium diets. Conversely, in rats fed the high-sodium diet, renal pelvic perfusion of ANG II suppresses the ARNA responses to increased renal pelvic pressure to levels similar to those in vehicle-treated rats fed the low-sodium diet. Taken together, these findings suggest that endogenous ANG II modulates the sensitivity of the renal mechanosensory nerve fibers by inhibiting the PGE_2-mediated release of substance P from the sensory nerve terminals in the renal pelvic wall (Fig. 10). Enhanced activation of the renorenal reflexes may contribute to the increased urinary sodium excretion during excess sodium intake.

Perspectives

Activation of the renorenal reflexes results in decreased ERNA (27). During conditions of sodium retention, the inhibitory effect of ANG II on ARNA would suppress the inhibitory renorenal reflexes, which would contribute to the increased ERNA generally attributed to an effect of ANG II in the central nervous system (2, 9, 11). The modulatory effect of the renin-angiotensin system on the renorenal reflexes further suggests that activation of the afferent renal nerves is an important factor in the spectrum of mechanisms activated to regulate water and sodium homeostasis. During excess dietary sodium intake, the enhanced activation of the renorenal reflexes would facilitate the excretion of an increased sodium load. In this context, it is of interest that rats inbred for low urinary kal-likrein excretion or mutant mice lacking bradykinin B_2 receptors develop hypertension when fed a high-sodium diet (1, 34), bradykinin being an important mediator in the activation of the renal pelvic mechanosensory nerves (25) (Fig. 10). Conversely, mice over-expressing B_2 receptors are hypertensive when they are fed a regular-sodium diet (50). Furthermore, in rats treated with capsaicin neonatally to destroy the sensory nerves, the natriuretic response to volume expansion is impaired (35). Moreover, a high-sodium diet increases arterial blood pressure in capsaicin-treated rats (49).

The potential importance of renorenal reflexes in contributing to increased urine output during conditions of excess sodium intake is further suggested by our findings that the responsiveness of the renal sensory nerves is impaired in spontaneously hypertensive rats (SHR) (22) and in a salt-sensitive hypertensive backcross population [F_1 × Wistar-Kyoto (WKY)], where F_1 = SHR × Wistar-Kyoto (10). The impairment of the renorenal reflexes in SHR is due to suppressed renal pelvic release of substance P (22). In this context, it is interesting to note that there is an increased renal responsiveness to activation of the renin-angiotensin system in young SHR (5, 7).

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REFERENCES


