Differential effects of endothelin on activation of renal mechanosensory nerves: stimulatory in high-sodium diet and inhibitory in low-sodium diet

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THE MAJORIT Y OF THE AFFERENT renal nerves are located in the renal pelvic wall (29, 35, 42). These nerves are activated by increases in renal pelvic pressure within the physiological range (32). The increase in afferent renal nerve activity (ARNA) produced by the increased renal pelvic pressure leads to a reflex decrease in efferent renal sympathetic nerve activity (ERSNA) and a diuresis and natriuresis, i.e., a renorenal reflex response (37). Together with other cardiovascular reflexes, e.g., the aortic and carotid baroreceptor reflexes, activation of the inhibitory renorenal reflexes contributes to the maintenance of low basal ERSNA.

Among the various mechanisms activated by stretching the renal pelvic wall is activation of bradykinin-2 receptors, leading to activation of protein kinase C and induction of cyclooxygenase-2 (COX-2), which in turn results in increased renal pelvic synthesis of PGE2 (34, 36). PGE2 stimulates EP4 receptors on or close to the renal sensory nerves, leading to activation of the cAMP-protein kinase A transduction pathway and a Ca2+-dependent release of the neuropeptide substance P (29, 33). Substance P activates the afferent renal nerves by stimulating neurokinin-1 receptors in the renal pelvic area (38).

The responsiveness of the afferent renal nerves is enhanced by high and suppressed by low-sodium dietary intake because of an interaction between PGE2 and angiotensin (ANG) II at the peripheral sensory nerve endings (30, 32, 33). In conditions of low-sodium dietary intake, high endogenous ANG II activity reduces the PGE2-mediated activation of adenyl cyclase via a pertussis toxin (PTX)-sensitive mechanism (30), leading to an impairment of the renorenal reflexes. Conversely, in conditions of high-sodium dietary intake, characterized by low endogenous ANG II (8), there is little or no inhibition of the PGE2-mediated activation of adenyl cyclase. The increased responsiveness of the afferent renal nerves in conditions of high-sodium dietary intake suggests that the renorenal reflex mechanism contributes to total body sodium and fluid volume balance by facilitating the excretion of an ingested sodium load. This hypothesis was subsequently confirmed by our studies in dorsal rhizotomized rats. Interrupting the afferent renal nerve input to the spinal cord at T9-L1 resulted in salt-sensitive hypertension (31). Dorsal root ganglia (DRG) at T9-L1 contain the majority of the cell bodies of the afferent renal nerves (10, 68).

There are many models of salt-sensitive hypertension, including the bradykinin-2 receptor-deficient mouse (2), rats fed an essential fatty acid-deficient diet (5), and rats with chronic renal medullary COX-2 inhibition (67). Interestingly, the mechanisms that have been modified to render these animals hypertensive when fed high-salt diet are involved in the activation of renal sensory nerves (34, 36). Also, in view of the inhibitory effect of ANG II on the renorenal reflexes (30), it is noteworthy that arterial pressure is increased in response to chronic administration of a low dose of ANG II when rats are fed high, but not when they are fed normal sodium dietary intake (23).

Another model of salt-sensitive hypertension is the endothelin (ET) B receptor-deficient rat (15, 47). ET is abundantly expressed throughout the body, including the brain and the kidney (18). ET exerts its effects by activating two G protein-coupled receptors, ETA and ETB (51). The responses to ET vary with the cell type/organ. The major vascular effects of
ET<sub>A</sub> receptor (ET<sub>A</sub>R) activation are vasoconstriction. The results of activation of ET<sub>B</sub> receptors (ET<sub>B</sub>R) are more diverse, including vasoconstriction, vasodilation, diuresis, and natriuresis (51). In the kidney, ET-1 is widely distributed, with the highest concentration in inner medulla (51, 61). Whereas ET<sub>A</sub>R are predominantly localized to the renal vasculature, ET<sub>B</sub>R are found in glomeruli, inner medullary collecting duct cells, and the renal pelvic area (69).

The mechanisms involved in salt-sensitive hypertension in the ET<sub>B</sub>R-deficient rat are not completely understood. A role for ET<sub>A</sub>R in the increased arterial pressure has been suggested by studies showing marked reduction of the hypertension by ET<sub>A</sub>R antagonists (15, 47). A possible role for ET-1 in the regulation of blood pressure via the sensory nerves was suggested by studies in rats treated neonatally with capsaicin to destroy all sensory nerves (64). These rats develop salt-sensitive hypertension that is reduced by ET<sub>A</sub>R antagonists in a fashion similar to that in ET<sub>B</sub>R-deficient rats (15, 47).

The expression of ET-1 together with preprotrychkinin-mRNA and CGRP mRNA in lumbar DRG (16) suggests the presence of ET-1 in sensory nerves. ET<sub>A</sub>R and ET<sub>B</sub>R have been localized on or close to central and peripheral sensory nerves (48). ET has been shown to activate nociceptors (e.g., Refs. 6, 25, 26), and several studies have indicated a dual effect of ET-1 on pain via activation of ET<sub>A</sub>R and ET<sub>B</sub>R (3, 25, 43).

The known modulatory role of ET-1 on nociceptors (6, 25, 26) and baroreceptors (4, 22, 40), together with the salt-sensitive hypertension in afferent renal denervated rats (31), suggests that an impairment of the renal reflexes may contribute to the salt-sensitive hypertension in ET<sub>B</sub>R-deficient rats. Therefore, we examined the role of ET-1 on the activation of renal mechanosensory nerves by studying the effects of ET<sub>B</sub>R and ET<sub>A</sub>R antagonists on the activation of renal mechanosensory nerves in conditions of normal, low, and high dietary sodium intake.

**METHODS**

The study was performed on male Sprague-Dawley rats weighing 176–380 g (mean: 291 ± 3 g). Two weeks before the study, the rats were placed on either sodium (Na<sup>+</sup>)-deficient pellets (ICN; Na<sup>+</sup> = 1.6 meq/kg) with tap water drinking fluid (low-sodium diet, n = 75), normal Na<sup>+</sup> pellets (Teklad; Na<sup>+</sup> = 163 meq/kg) with tap water drinking fluid (normal sodium diet, n = 28), or normal Na<sup>+</sup> pellets with 0.9% NaCl drinking fluid (high-sodium diet, n = 70) (32). The experimental protocols were approved by the Institutional Animal Care and Use Committee and performed according to the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg ip; Abbott Laboratories).

**Renal and DRG Tissue ET-1 Concentrations**

The renal pelvis, medulla, and cortex and DRG (T<sub>9</sub>-L<sub>1</sub>) were dissected from rats fed low (n = 10) and high (n = 11) sodium diet and immediately placed on dry ice. The tissue was stored at −80°C for later analysis of ET-1 concentration.

**In Vivo Studies**

After induction of anesthesia (see above), an intravenous infusion of pentobarbital sodium (0.04 mmol·kg<sup>−1</sup>·h<sup>−1</sup>) at 50 μl/min into the femoral vein was started and continued throughout the course of the experiment. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA have been previously described in detail (29–38). In brief, the left renal pelvis was perfused with vehicle or various perfusates, described below, throughout the experiment at 20 μl/min via a PE-10 catheter placed inside a PE-60 catheter located in the ureter. Renal pelvic pressure was increased by elevating the fluid-filled ureteral catheter above the level of the kidney. A PE-10 catheter was inserted into the right contralateral ureter for collection of urine. ARNA was recorded from the peripheral portion of the cut end of one left renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-s intervals, with a unit of measure of microvolts per second per second. Postmortem renal nerve activity, 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.

**Experimental Protocol**

The studies were divided into three main groups, groups I–III. In group I rats, we examined the effects of an ET<sub>B</sub>R and an ET<sub>A</sub>R antagonist on the ARNA responses to increasing renal pelvic pressure in rats fed high-, normal, and low-sodium diet. In group II rats, we examined whether an ET<sub>A</sub>R antagonist blocked the effects produced by an ET<sub>B</sub>R antagonist on the ARNA responses in rats fed high-sodium diet. We also examined whether an ET<sub>B</sub>R antagonist blocked the effects of an ET<sub>A</sub>R antagonist on the ARNA responses in rats fed low-sodium diet. In group III rats, which served as time controls, renal pelvic pressure was increased in the absence of any ET receptor (ETR) antagonists.

In each group, renal pelvic pressure was increased 2.5 or 7.5 mmHg during a 5-min experimental period, as detailed below. Each experimental period was bracketed by a 10-min control and a 10-min recovery period.

**Group I A: Effects of an ET<sub>B</sub>R antagonist on ARNA responses to increased renal pelvic pressure.** Rats were fed either high-sodium diet (n = 10) or normal sodium diet (n = 6). The experiment was divided into two parts. During each part, renal pelvic pressure was increased 2.5 and 7.5 mmHg during two experimental periods. After the end of the first part, the renal pelvic perfusate was switched from vehicle to the ET<sub>B</sub>R antagonist BQ-788 (21) (1 μM). Ten minutes later, the two control, experimental, and recovery periods were repeated.

**Group I B: Effects of an ET<sub>A</sub>R antagonist on ARNA responses to increased renal pelvic pressure.** Rats were fed either low-sodium diet (n = 8) or normal sodium diet (n = 6). The experimental protocol was similar to that for group I A, except the renal perfusate was perfused with the ET<sub>A</sub>R antagonist BQ-123 (20). Because pilot experiments showed inconsistent effects of BQ-123 at 1 μM, the studies were performed using BQ-123 at 5 μM.

**Group II: Effects of an ET<sub>B</sub>R and an ET<sub>A</sub>R antagonist alone and in combination on ARNA responses to increased renal pelvic pressure.** Rats were fed high- or low-sodium diet (n = 8) or normal sodium diet (n = 6). The experimental protocol was similar to that for group I A, except the renal perfusate was perfused with the ET<sub>A</sub>R antagonist BQ-123 (20). Because pilot experiments showed inconsistent effects of BQ-123 at 1 μM, the studies were performed using BQ-123 at 5 μM.

**Group III: Effects of an ET<sub>B</sub>R and an ET<sub>A</sub>R antagonist alone and in combination on ARNA responses to increased renal pelvic pressure.** Rats were fed high- or low-sodium diet (n = 8) or normal sodium diet (n = 6). The experimental protocol was similar to that for group I A, except the renal perfusate was perfused with the ET<sub>A</sub>R antagonist BQ-123 (20). Because pilot experiments showed inconsistent effects of BQ-123 at 1 μM, the studies were performed using BQ-123 at 5 μM.

The renal pelvis, medulla, and cortex and DRG (T<sub>9</sub>-L<sub>1</sub>) were dissected from rats fed low (n = 10) and high (n = 11) sodium diet and immediately placed on dry ice. The tissue was stored at −80°C for later analysis of ET-1 concentration.

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The renal pelvis, medulla, and cortex and DRG (T<sub>9</sub>-L<sub>1</sub>) were dissected from rats fed low (n = 10) and high (n = 11) sodium diet and immediately placed on dry ice. The tissue was stored at −80°C for later analysis of ET-1 concentration.

**In Vivo Studies**

After induction of anesthesia (see above), an intravenous infusion of pentobarbital sodium (0.04 mmol·kg<sup>−1</sup>·h<sup>−1</sup>) at 50 μl/min into the femoral vein was started and continued throughout the course of the experiment. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA...
Group III: Increasing renal pelvic pressure in the absence of ETR antagonists (time controls). Rats were fed a high-sodium diet (n = 10) or a low-sodium diet (n = 10). The experimental protocol was similar to that in group II, except the renal pelvis was perfused with vehicle throughout the experiment.

In Vitro Studies

Substance P release from an isolated renal pelvic wall preparation. To examine whether the effects of ET_{A}R and ET_{B}R antagonists on ARNA were related to a mechanism(s) at the peripheral sensory nerves endings and independent of any possible systemic and/or central effects, we examined the effects of the ETR antagonists on the PGE_{2}-mediated release of substance P using an isolated renal pelvic wall preparation.

The procedures for stimulating the release of substance P from an isolated rat renal pelvic wall preparation have been previously described in detail (30, 32, 35, 36). In brief, after anesthesia, renal pelvises dissected from the kidneys were placed in wells containing 400 μl of HEPES (25 mM HEPES, 135 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl_{2}, 1 mM MgCl_{2}, 3.3 mM d-glucose, 0.1 mM ascorbic acid, 0.1% BSA, 10 μM dl-thiopropanol, 1 mM Phe-Ala, and 50 μM p-chloromercuriphenylsulfonic acid, pH 7.4) maintained at 37°C. Indomethacin (0.14 mM) was present in the incubation bath to minimize the influence of endogenous PGE_{2} on substance P release. Each well contained the pelvic wall from one kidney.

The renal pelvic walls were allowed to equilibrate for 130 min. The incubation medium was replaced with fresh HEPES every 10 min for the first 120 min and every 5 min thereafter. The incubation medium was replaced with siliconized vials and stored at −80°C for later analysis of substance P. The experimental protocol consisted of four 5-min control periods, one 5-min experimental period, and four 5-min recovery periods. PGE_{2} was added to the incubation bath for both the ipsilateral and contralateral pelvises during the experimental periods.

In vitro study was divided into two main groups, groups IV and V. In group IV, we examined whether stimulating the renal pelvic wall with an ET_{A}R or an ET_{B}R antagonist altered the PGE_{2}-mediated release of substance P into the incubation bath. The pelvises were derived from rats fed high-, normal-, or low-sodium diet. In group V, we compared the effects of BQ-788 and BQ-123 alone and in combination on PGE_{2}-mediated release of substance P.

The various concentrations of PGE_{2} used in groups IV and V represent those that are subthreshold for substance P release in rats fed a high-, normal-, or low-sodium diet, 0.014, 0.03, or 0.14 μM, respectively, and those that are required to produce an increase in substance P release in rats fed a high- and normal sodium diet, 0.03 and 0.14 μM, respectively (32).

Group IVA: Effects of an ET_{A}R antagonist on PGE_{2}-mediated release of substance P. Rats were fed high- or normal sodium diet. The ipsilateral renal pelvis was incubated in HEPES/indomethacin buffer as described above. The contralateral renal pelvis was incubated in HEPES/indomethacin buffer containing BQ-788 (1 μM) throughout the control, experimental, and recovery periods. During the experimental period, the ipsilateral and contralateral pelvises were exposed to PGE_{2} at 0.03 μM in the high-sodium diet group (n = 11) and to PGE_{2} at 0.14 μM in the normal sodium diet group (n = 8).

Group IVB: Effects of an ET_{B}R antagonist on PGE_{2}-mediated release of substance P. Rats were fed high-, normal, or low-sodium diet. The first group was fed a high-sodium diet (n = 6), the second (n = 8) and third groups (n = 8) a normal sodium diet, and the fourth group a low-sodium diet (n = 15). The experimental protocol was similar to that described above, except the contralateral pelvis was incubated in HEPES/indomethacin buffer containing BQ-123 throughout the experiment. Because our initial experiments in the low-sodium diet group showed similar effects produced by 1 and 5 μM BQ-123, all subsequent in vitro studies used BQ-123 at 1 μM.

During the experimental period, the ipsilateral and contralateral pelvises were exposed to PGE_{2} at 0.014 (n = 3) and 0.03 μM (n = 6) in rats fed a high-sodium diet and to PGE_{2} at 0.14 μM in rats fed a low-sodium diet (n = 15). Pelvises from rats fed a normal sodium diet were exposed to PGE_{2} at either 0.03 (n = 8) or 0.14 μM (n = 8).

Group V: Effects of an ET_{A}R and an ET_{B}R antagonist alone and in combination on PGE_{2}-mediated release of substance P. Rats were fed a high-sodium diet (n = 14) or a low-sodium diet (n = 13). Ipsilateral pelvises from rats fed high- and low-sodium diets were incubated in HEPES/indomethacin buffer containing BQ-788 (1 μM) and BQ-123 (1 μM), respectively. The contralateral pelvises from either group were incubated in HEPES/indomethacin buffer containing BQ-788 plus BQ-123, both at 1 μM. During the experimental period, PGE_{2} at 0.03 and 0.14 μM was added to both pelvises from the rats fed high- and low-sodium diets, respectively.

RESULTS

Renal and DRG Tissue ET-1 Concentrations

As shown in Fig. 1, ET-1 is present in renal pelvic tissue at a concentration 15 times lower than that in papillary tissue but 10 times higher than that in cortical tissue. ET-1 was also present in tissue from DRG at T_{9}-L_{1}. ET-1 levels were similar in rats fed high- and low-sodium diet in both renal and DRG tissues.
In Vivo Studies

**Group IA:** Effects of an ET\(_{B}\)R antagonist on ARNA responses to increased renal pelvic pressure. The presence of ET-1 in renal pelvic tissue and DRG (T\(_9\)-L\(_1\)) (Fig. 1) and ET\(_{B}\)R in renal pelvic wall (69) suggested that activation of ETB-R might modulate renal sensory nerves. As shown in Fig. 2, renal pelvic perfusion with the ET\(_{B}\)R antagonist BQ-788 suppressed the ARNA responses to increasing renal pelvic pressure in rats fed high, but not in rats fed normal sodium dietary intake. The increases in contralateral urinary sodium excretion produced by increased renal pelvic pressure reached statistical significance in response to an increase in renal pelvic pressure of 7.5 mmHg during vehicle perfusion in the rats fed a high-sodium diet, with urinary sodium excretion increased from 1.0 \(\pm\) 0.2 to 1.3 \(\pm\) 0.2 \(\mu\)mol\(\cdot\)min\(^{-1}\cdot\)g\(^{-1}\) \((P < 0.01)\). Mean arterial pressure remained unaltered throughout the experiments, being 114 \(\pm\) 3 and 108 \(\pm\) 3 mmHg in rats fed high- and normal sodium diets, respectively.

**Group IB:** Effects of an ET\(_{A}\)R antagonist on ARNA responses to increased renal pelvic pressure in rats. Because of the well-documented effects of ET\(_{A}\)R activation on nociceptors, we speculated that an ET\(_{A}\)R antagonist might modulate the activation of renal sensory nerves, albeit the increases in renal pelvic pressure required to increase ARNA is below that for sensation of pain (32). As shown in Fig. 3, renal pelvic perfusion with the ET\(_{A}\)R antagonist BQ-123 produced a significant enhancement of the ARNA responses to an increase in renal pelvic pressure of 2.5 and 7.5 mmHg in rats fed low- but not in rats fed normal sodium dietary intake. Mean arterial pressure remained unaltered throughout the experiments in both groups of rats, being 114 \(\pm\) 2 and 115 \(\pm\) 6 mmHg in rats fed low- and normal sodium diets, respectively.

**Group II:** Effects of an ET\(_{B}\)R and an ET\(_{A}\)R antagonist alone and in combination on ARNA responses to increased renal pelvic pressure. The results in group IB together with those showing that an ET\(_{A}\)R antagonist reduced arterial pressure...
in ET_{BR}-deficient rats fed high-sodium diet (15, 47) suggested that the suppressed ARNA response to increased renal pelvic pressure in the presence of ET_{BR} blockade in high-sodium diet rats may be due to activation of ET_{AR}. Therefore, in rats fed a high-sodium diet, we examined whether an ET_{AR} antagonist would restore the BQ-788-induced reduction of the ARNA responses to increased renal pelvic pressure toward control responses. As shown in Fig. 4, and similar to our findings in group IA (Fig. 2), renal pelvic perfusion with BQ-788 produced a marked reduction of the ARNA response to an increase in renal pelvic pressure of 7.5 mmHg. Adding BQ-123 to the renal pelvic perfusate containing BQ-788 restored the ARNA response toward its response in the presence of vehicle. Continued renal pelvic perfusion with BQ-788 without the addition of BQ-123 resulted in a maintained suppression of the ARNA responses to increased renal pelvic pressure (Table 1). Mean arterial pressure at 118 ± 3 and 123 ± 5 mmHg in the two groups remained unaltered throughout the experiment.

Because these studies suggested that the impaired responsiveness of the renal mechanosensory nerves following ET_{BR} blockade was due to ET-1 activating ET_{AR} (Fig. 4), we examined whether a similar interaction between the two ETRs occurred in conditions of low-sodium dietary intake. As shown in Fig. 4, and similar to our findings in group IB (Fig. 3), renal pelvic perfusion with BQ-123 enhanced the ARNA response to an increase in renal pelvic pressure of 7.5 mmHg. Adding BQ-788 to the renal pelvic perfusate containing BQ-123 suppressed the ARNA response toward that seen during vehicle perfusion. Continued renal pelvic perfusion with BQ-123 without the addition of BQ-788 resulted in similar enhanced ARNA responses to increased renal pelvic pressure (Table 1). Mean
In Vitro Studies

Table 1. ARNA responses to increasing renal pelvic pressure in the presence of vehicle and BQ-788 in rats fed a high-sodium diet and vehicle and BQ-123 in rats fed a low-sodium diet

<table>
<thead>
<tr>
<th>Renal Pelvic Perfusion</th>
<th>ARNA, %</th>
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<tbody>
<tr>
<td><strong>High-sodium diet</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>23±4*</td>
</tr>
<tr>
<td>BQ-788</td>
<td>3±3†</td>
</tr>
<tr>
<td>BQ-788</td>
<td>9±2†</td>
</tr>
<tr>
<td><strong>Low-sodium diet</strong></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7±2*</td>
</tr>
<tr>
<td>BQ-123</td>
<td>27±7†</td>
</tr>
<tr>
<td>BQ-123</td>
<td>29±6†</td>
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</tbody>
</table>

Afferent renal nerve activity (ARNa) responses to increasing renal pelvic pressure 7.5 mmHg three times in the presence of vehicle and BQ-788 in rats fed a high-sodium diet and in the presence of vehicle and BQ-123 in rats fed a low-sodium diet. Values are means ± SE; n = 6 in both groups. *P < 0.01 vs. baseline. †P < 0.01 vs. ARNA response during vehicle perfusion.

Among the mechanisms involved in the activation of the afferent renal nerves during an increase in renal pelvic pressure, it is increased renal pelvic PGE2 synthesis, leading to a release of substance P (36). To examine whether the modulatory effects of ET-1 on ARNA (Figs. 2–4) were due to ET-1 activating a peripheral mechanism at the sensory nerve endings in the renal pelvic wall, we examined the effects of incubating isolated renal pelvises with BQ-788 and BQ-123 on the PGE2-mediated release of substance P.

Group IVA: Effects of an ETaR antagonist on PGE2-mediated release of substance P. As shown in Fig. 5 and similar to our previous studies (32), substance P was released into the incubation bath by PGE2 at a concentration five times lower in rats fed a high-sodium diet (0.03 μM) than a normal sodium diet (0.14 μM). Adding BQ-788 to the bath markedly suppressed the PGE2-mediated substance P release from pelvises derived from rats fed a high-sodium diet but had no effect on the substance P release from pelvises derived from rats fed a normal sodium diet.

Group IVB: Effects of an ETaR antagonist on PGE2-mediated release of substance P. As shown in Fig. 6 and in agreement with our previous studies (32), PGE2 at a concentration that increased substance P release from pelvises derived from rats fed normal sodium diet failed to increase substance P release from pelvises derived from rats fed low-sodium diet. Adding BQ-123 at 5 or 1 μM to the bath containing the pelvises of rats fed low-sodium diet enhanced the PGE2-mediated substance P release to the same extent, so the data have been pooled. BQ-123 had no effect on the substance P release produced by PGE2 (0.14 μM) in rats fed normal sodium diet. Further studies in pelvises from rats fed normal sodium diet showed that BQ-123 produced a small enhancement of the substance P release produced by a subthreshold concentration of PGE2 (0.03 μM), with the magnitude of the substance P release being less than that produced from pelvises derived from rats fed low-sodium diet treatments BQ-123 (P < 0.05). On the other hand, in rats fed high-sodium diet, BQ-123 had no effect on renal pelvic release of substance P produced by PGE2 at 0.03 μM (Table 3) or 0.014 μM (subthreshold concentration for substance P release in rats fed high-sodium diet): vehicle-treated pelvises: from 8.8 ± 5.9 to 11.4 ± 7.7 pg/min; BQ-123-treated pelvises, from 6.3 ± 3.0 to 6.3 ± 2.4 pg/min (n = 3).

Group V: Effects of an ETaR antagonist and an ETaR antagonist alone and in combination on PGE2-mediated release of substance P. Comparing the PGE2-mediated release of substance P from ipsilateral and contralateral pelvises from high-sodium diet rats treated with BQ-788 and BQ-788 plus BQ-123 (Figs. 5 and 7) showed that the BQ-788-mediated suppression of the PGE2-mediated release of substance P was prevented by incubating the pelvises in a combination of BQ-788 and BQ-123. Comparing the PGE2-mediated release of substance P from ipsilateral and contralateral pelvises from low-sodium diet rats incubated with BQ-123 and BQ-123 plus BQ-788 (Figs. 6 and 7) showed that the BQ-123-mediated enhancement of the PGE2-mediated release of substance P was not observed when the pelvises were incubated in a combination of BQ-123 and BQ-788.

**DISCUSSION**

The results of these experiments show that ET-1 is present in renal pelvic and DRG (T9-L1) tissues. Renal pelvic administration of an ETαR antagonist suppresses the PGE2-mediated release of substance P and the ARNA response to increased renal pelvic pressure in conditions of high-sodium dietary

Table 2. ARNA responses to increasing renal pelvic pressure in the presence of vehicle in rats fed high- and low-sodium diets

<table>
<thead>
<tr>
<th>Renal Pelvic Perfusion</th>
<th>High-Sodium Diet (n=10)</th>
<th>Low-Sodium Diet (n=10)</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>20±4†</td>
<td>10±2*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>25±4†</td>
<td>11±4*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>31±5†</td>
<td>15±3‡</td>
</tr>
</tbody>
</table>

**ARNA responses to increasing renal pelvic pressure 7.5 mmHg three times in the presence of vehicle were measured in rats fed high- and low-sodium diets (time controls).** Values are means ± SE; n = 10 in both groups. *P < 0.05, †P < 0.01 vs. baseline.
intake. Conversely, in conditions of low-sodium dietary intake, renal pelvic administration of an ET$_A$R antagonist enhances the PGE$_2$-mediated release of substance P and the ARNA response to increased renal pelvic pressure. In conditions of normal sodium dietary intake, the activation of renal mechanosensory nerves is slightly enhanced by an ET$_A$R antagonist but is not affected by an ET$_B$R antagonist. Together, these data suggest that ET plays a powerful modulatory role in the activation of renal pelvic mechanosensory nerves, the nature of which is dependent on dietary sodium intake.

Whereas ET-1 mRNA and ET$_A$R are expressed in sensory nerve bodies in lumbar DRG, ET$_B$R have been located to glia cells surrounding central and peripheral sensory nerves (16, 48), suggesting that ET-1 may modulate peripheral sensory nerves by activating ET$_A$R located on the sensory nerve fibers and ET$_B$R on peripheral glia cells surrounding sensory nerve fibers. These studies, together with the presence of ET-1 in renal pelvic tissue and DRG (T$_9$-L$_1$) (current studies) and ET$_B$R in the renal pelvic wall (69), suggest that ET-1 modulates renal pelvic sensory nerves by activating its receptors on or close to sensory nerve fibers.

The majority of studies examining the effects of ET-1 as a modulator of sensory nerve activity have been focused on its role as a mediator of pain. However, ET-1 has also been shown to modulate the carotid baroreceptor reflex. Central administration of ET-1 and ET-3, the latter having higher affinity for ET$_B$R than ET$_A$R (51), was shown to increase baroreflex sensitivity (22), whereas local administration of ET-1 into an isolated baroreceptor preparation was shown to suppress the baroreceptor activity (4, 40), possibly via activation of ET$_A$R (40).

Renal Mechanosensory Nerve Activation: Role of ET-Mediated Activation of ET$_B$R and ET$_A$R

The threshold pressure for activation of renal pelvic mechanosensory nerves of $<2.5$ mmHg in rats fed a high-sodium diet.
renal pelvic administration of the ETBR antagonist BQ-788

isolated renal pelvises incubated in vehicle or BQ-123

The responses to activation of ETBR vary with the ET-1 mouse develop salt-sensitive hypertension (1, 15, 47), pressure. Importantly, the ETBR antagonist had no effect on the suppressed the ARNA responses to increased renal pelvic density of ETBR (24, 51, 65).

inner medullary collecting duct cells, which contain a high increasing sodium excretion by an effect, at least in part, on natriuresis (51). In vitro studies have shown evidence for ET-1 tissue/organ and include vasoconstriction, vasodilation, and

load. The responses to activation of ETBR are characterized by a normal sodium diet, a condition characterized by no or

diet (32) suggests that the renal mechanosensory nerves are tonically active in conditions of high-sodium dietary intake. This idea is supported by studies showing that unilateral renal denervation produces a renorenal reflex increase in contralateral ERSNA and a decrease in contralateral urinary sodium excretion in volume-expanded rats (9). Also, selective bilateral afferent renal denervation results in increased arterial pressure in rats fed high-, but not normal, sodium dietary intake (31).

There is considerable evidence for ET playing an important role in the maintenance of water and sodium balance. The ETBR-deficient rat and the collecting duct-specific knockout of ET-1 mouse develop salt-sensitive hypertension (1, 15, 47), presumably to facilitate the excretion of an increased sodium load. The responses to activation of ETBR vary with the tissue/organ and include vasoconstriction, vasodilation, and natriuresis (51). In vitro studies have shown evidence for ET-1 increasing sodium excretion by an effect, at least in part, on inner medullary collecting duct cells, which contain a high density of ETBR (24, 51, 65).

The present studies in rats fed high-sodium diet show that renal pelvic administration of the ETBR antagonist BQ-788 suppressed the ARNA responses to increased renal pelvic pressure. Importantly, the ETBR antagonist had no effect on the responsiveness of the renal mechanosensory nerves in rats fed a normal sodium diet, a condition characterized by no or minimal tonic activation of renal sensory nerves (32). In view of the inhibitory nature of the renorenal reflexes (37), it is interesting that the ET-1-deficient mouse is characterized by increased ERSNA (41). Together, these studies suggest that an impaired responsiveness of the renal mechanosensory nerves in the ETBR-deficient rat contributes to the salt-sensitive hypertension.

In conditions of low-sodium dietary intake, when endogenous ANG II is increased (8), the activation threshold of the renal mechanosensory nerves is above basal renal pelvic pressure (32), suggesting a tonic suppression of the natriuretic renorenal reflexes in conditions characterized by sodium retention. Our previous studies have shown an important role for endogenous ANG II in mediating the impaired responsiveness of renal sensory nerves. There is considerable evidence for a interaction between ANG II and ET-1 (28, 49, 52, 62, 66). Nonneural cardiovascular and renal responses to ANG II are reduced by an ETAR antagonist (11, 50), suggesting that ET-1, by activating ETAR, may contribute at least in part to some of the effects produced by ANG II in cardiac and renal tissue. We therefore hypothesized that an ETAR antagonist may enhance the responsiveness of the renal mechanosensory nerves in a fashion similar to that of an AT-1 receptor antagonist (32). Our findings supported our hypothesis. In conditions of low-sodium diet, renal pelvic administration of the ETAR antagonist BQ-123 enhanced the ARNA responses to increased renal pelvic pressure. In rats fed a normal sodium diet, the ETAR antagonist had no significant effect on the ARNA responses to increasing renal pelvic pressure below or above the threshold for activation of renorenal mechanosensory nerves, i.e., 2.5 and 7.5 mmHg. Together, our findings suggest that ET-1 via activation of ETAR contributes to the suppression of the responsiveness of renal pelvic mechanosensory nerves in conditions of low-sodium dietary intake.

To explore where in the chain of events leading to increased ARNA following increased renal pelvic pressure ET-1 may exert its effect, we turned to the isolated renal pelvic wall preparation to examine whether the ETR antagonists would involve mechanisms before and/or after the increased renal pelvic PGE2 synthesis produced by stretching the pelvic wall. By design, this experimental model also excludes systemic and central mechanisms that may modulate the activation of renal

Table 3. PGE2-mediated release of substance P from isolated renal pelvises incubated in vehicle or BQ-123

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Substance P Release, pg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (Vehicle)</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td>BQ-123</td>
<td>8.5 ± 2.2</td>
</tr>
</tbody>
</table>

Pelvises were derived from rats fed high-sodium diet. Values are means ± SE; n = 6. *P < 0.01 vs. average of control and recovery.

Fig. 7. A: rats fed a high-sodium diet, effects of PGE2 at 0.03 μM on substance P release from an isolated renal pelvic wall preparation incubated in BQ-788 or BQ-788 + BQ-123. B: rats fed a low-sodium diet, effects of PGE2 at 0.14 μM on substance P release from an isolated renal pelvic wall preparation incubated in BQ-123 or BQ-123 + BQ-788. **P < 0.01 vs. control and recovery values. ‡P < 0.01 vs. BQ-788 and BQ-123 + BQ-788 in rats fed high- and low-sodium diet, respectively.
sensory nerves. Our data showing that the PGE2-mediated release of substance P was suppressed by acute administration of an ETB antagonist to renal pelvises derived from rats fed a high-sodium diet and enhanced by acute administration of an ETA antagonist to renal pelvises derived from rats fed a low-sodium diet, suggest that ET-1 modulates the PGE2-mediated activation of renal sensory nerves by a mechanism(s) at the peripheral sensory nerve terminals.

BQ-123 and BQ-788 are selective ETA and ETB antagonists with no agonist activity at the concentrations used (20, 21). To examine whether the effects produced by the two ETR antagonists were specific to each antagonist and the dietary sodium intake, we examined the effects of BQ-788 and BQ-123 in rats fed various sodium diets. Whereas BQ-788 suppressed the responsiveness of the renal sensory nerves in rats fed a high-sodium diet both in vivo and in vitro, BQ-788 had no effect in rats fed a normal sodium diet. Conversely, BQ-123 enhanced the responsiveness of the renal mechanosensory nerves in rats fed a low- and normal sodium diet, with the enhancement being greater in rats fed a low- than normal sodium diet. Importantly, BQ-123 neither enhanced nor suppressed the PGE2-mediated substance P release in rats fed a high-sodium diet. Together, these findings suggest that the effects produced by BQ-788 and BQ-123 were specific to the ETB and ETA, respectively, and to the various dietary sodium intakes.

**Activation of Renal Mechanosensory Nerves: Interaction Between ETB and ETA**

Studies in ETB-deficient rats have shown that activation of ETA contributes to the salt-sensitive hypertension (15, 47). Likewise, in vivo and in vitro studies in rats fed high-sodium diet show that the impaired responsiveness of the renal mechanosensory nerves after renal pelvic administration of the ETB antagonist was due to activation of ETA, with the activation of ETA revealed by blocking the ETB. The enhanced activation of ETA in the absence of functioning ETB has been suggested to involve increased plasma concentrations of ET-1, due to the ETB being a clearance receptor for circulating ET-1 (14). Although this mechanism may explain the increased activation of renal mechanosensory nerves produced by the ETA antagonist following ETB blockade in vivo, it is unlikely that this mechanism would explain the enhanced effect of the ETA antagonist on the PGE2-mediated release of substance P in vitro. In preliminary studies, we were unable to show increased ET-1 concentration in renal pelvic tissue after incubation with BQ-788 (22.2 ± 4.1 pg/mg protein) compared with vehicle (17.8 ± 2.0 pg/mg protein). Because the marked effects of the ET-1-mediated ETA activation were observed after acute ETB blockade, it is not likely that they are due to increased ETB expression, which has been reported in mesenteric arteries of ETB-deficient rats (45). Our studies further show a similar interaction between the activation of ETB and ETA in rats fed a low-sodium diet. The enhanced responsiveness of the renal mechanosensory nerves in the presence of the ETA antagonist was due to activation of ETB at the peripheral renal pelvic sensory nerve endings.

**Dual Effects of ET-1: Role of Dietary Sodium Intake**

Our studies suggest a dual role for ET-1 in the activation of renal mechanosensory nerves, which is dependent on dietary sodium intake. The mechanisms involved in the differential effects of ET on renal mechanosensory nerves in rats fed various sodium dietary intakes are currently not known. ET-1 has a high affinity to both ETA and ETB (7, 51). Interestingly, numerous studies have also reported a dual control of pain-related actions of ET-1 (25, 43). The dual role for ET-1 in the activation of nociceptors exerting an algesic effect via activation of ETA and an analgesic effect by activating ETB (25) has been explained to be, at least in part, related to the ET-1 concentrations (6, 55). However, it is unlikely that the effects of ETB and ETA antagonists in rats fed high-, normal, and low-sodium diets can be explained by the renal and neural tissue ET-1 concentration being modulated by dietary sodium, because our studies showed similar ET-1 levels in renal pelvic and neural tissue in rats fed high- and low-sodium diets. Likewise, previous studies in rats and mice have failed to show increased ET-1 levels in renal cortical and medullary tissues in rats fed high-sodium diet (44, 52) despite increased urinary excretion of ET-1 (52) and increased expression of endothelin-converting enzyme in the renal medulla (13). The reasons for this apparent discrepancy are not clear. Although we cannot exclude the possibility that the differential effects of ET-1 in vivo could be related to urinary ET-1 excretion being higher in rats fed high- than in rats fed low-sodium diet, the similar nature of the responses to ETB

**Fig. 8.** In the sequence of events elicited during an increase in renal pelvic pressure, PGE2 exerts a stimulatory, and ANG II, an inhibitory effect on the activation of adenyl cyclase. The interaction between PGE2 and ANG II determines the level of activation of adenyl cyclase and, subsequently, the activation of the renal sensory nerves (30, 32, 33, 36). In view of the results from the present studies, we hypothesize that in conditions of high-sodium dietary intake, ET-1 modulates the responsiveness of renal sensory nerves via activation of ETB by enhancing the PGE2-mediated activation of adenyl cyclase. Conversely, in conditions of low dietary sodium intake, ET-1 via activation of ETA contributes to the ANG II-mediated suppression of the PGE2-induced activation of adenyl cyclase. PKC, protein kinase C; COX-2, cyclooxygenase-2; PKA, protein kinase A.
and ETAR antagonists in vivo and in vitro, respectively, does not support a role for urinary ET-1 concentrations, modulating the responsiveness of renal mechanosensory nerves. The incubation buffers were the same for renal pelvices derived from rats fed high-, normal, or low-sodium diets.

It is possible that the differential effects of ET on renal mechanosensory nerves could be explained by dietary sodium altering ETAR expression. Currently, there are few studies examining the effects of dietary sodium on ETAR expression, and the results appear to be somewhat inconsistent. Treating cell membrane from human renal medullary tissue in vitro with high sodium concentration increased the expression of ETAR but did not alter the ETAR expression (59). In DOCA-salt-sensitive hypertensive, there is an increased ETAR binding in renal medulla (46). However, whether this increase is due to salt loading, per se, or to the combination of DOCA and high-sodium diet was not reported in this study. In an apparent conflict with these studies is the increased human renal medullary expression of ETAR in conditions of low-sodium diet (59). The latter findings may be explained by a study in isolated proximal tubular cells, which showed that ANG II increased the expression of ETAR in renal proximal tubular cells (66). These findings appear to contradict the notion of ETAR activation leading to increased diuresis and natriuresis (51) but may represent a mechanism to buffer the increased tubular sodium reabsorption produced by angiotensin.

**Activation of Renal Mechanosensory Nerves: Possible Mechanisms Involved in Activation of ET-1 on ETAR and ETBR**

The current studies suggest that the effects of ETAR and ETBR activation on the responsiveness of renal sensory nerves involve an interaction between PGE2 and ET-1. The ETR have been shown to be coupled to multiple intracellular signaling mechanisms, depending on the cell type (55). ET-1 induces COX-2 expression (19) and increases PGE2 synthesis and activation of cAMP (54), possibly by activating ETAR (27) in nonneural renal tissue. ET-3, which has high affinity for ETBR (28) and ETAR antagonists in vivo and in vitro, respectively, does not support a role for urinary ET-1 concentrations, modulating the responsiveness of renal mechanosensory nerves. Because PG synthesis was inhibited in our in vitro studies, our data suggest that the ET-1-mediated enhancement of renal sensory nerves may involve a mechanism beyond the activation of EP4 receptors, at least in vitro (29). However, our data do not exclude the possibility that ET-1 may also enhance the responsiveness of the renal sensory nerves by increasing PGE2 synthesis in vivo. Conversely, in conditions of low-sodium diet, activation of ETAR may suppress PGE2-mediated activation of renal sensory nerves by preventing the PGE2-mediated activation of cAMP, possibly involving mechanisms activated by ANG II (Fig. 8).

The restoration of the responsive of the renal sensory nerves to baseline in the presence of a combination of ETAR and ETBR antagonists in conditions of high- and low-sodium diet suggests that the powerful modulatory role of ET-1, revealed by blocking only one of the ETRs, is one of several mechanisms involved in the activation of the renal sensory nerves.

In summary, the present study shows the presence of ET-1 in renal pelvic tissue and DRG (T9-L1), suggesting the presence of ET-1 in renal pelvic sensory nerves. The PGE2-mediated release of substance P and the increases in ARNA produced by increases in renal pelvic pressure within the physiological range were blocked by renal pelvic administration of an ETAR antagonist in rats fed high-sodium diet and enhanced by an ETAR antagonist in rats fed a low-sodium diet. These data suggest that ET in renal pelvic tissue has a dual effect on the activation of renal mechanosensory nerves. In conditions of high-sodium diet, ET by activating ETAR contributes to the suppressed responsiveness of renal mechanosensory nerves. Conversely, in conditions of low-sodium diet, ET by activating ETBR contributes to the suppressed responsiveness of the renal mechanosensory nerves. Interestingly, in conditions of normal sodium dietary intake, when the renorenal reflexes are neither suppressed nor enhanced, ET has no or minimal effect on renal mechanosensory nerve activation.

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