Activation of Endothelin-A Receptors Contributes to Angiotensin-Induced Suppression of Renal Sensory Nerve Activation

Ulla C. Kopp, Michael Z. Cicha, Lori A. Smith

Abstract—Activation of renal mechanosensory nerves is enhanced by a high-sodium diet and suppressed by a low-sodium diet. Angiotensin (Ang) II and endothelin (ET)-1 each contributes to the impaired responsiveness of renal mechanosensory nerves in a low-sodium diet. We examined whether stimulation of ETA receptors (Rs) contributes to Ang II–induced suppression of the responsiveness of renal mechanosensory nerves. In anesthetized rats fed a low-sodium diet, renal pelvic administration of the Ang type I receptor (AT1-R) antagonist losartan enhanced the afferent renal nerve activity (ARNA) response to increasing renal pelvic pressure 7.5 mm Hg from 7 ± 2% to 15 ± 2% and the prostaglandin (PG) E2–mediated substance P release from 0 ± 1 to 8 ± 1 pg/min. Adding the ETA-R antagonist BQ123 to the renal pelvic perfusate containing losartan did not produce any further enhancement of the ARNA response or PGE2–mediated release of substance P (17 ± 3% and 8 ± 1 pg/min). Likewise, renal pelvic administration of BQ123 and BQ123 + losartan resulted in similar enhancements of the ARNA responses to increased renal pelvic pressure and PGE2–mediated substance P release. In high-sodium–diet rats, pelvic administration of Ang II reduced the ARNA response to increased renal pelvic pressure from 27 ± 4% to 8 ± 3% and the PGE2–mediated substance P release from 9 ± 0 to 1 ± 1 pg/min. Adding BQ123 to the renal pelvic perfusate containing Ang II restored the increases in ARNA and the PGE2–mediated substance P release toward control (27 ± 6% and 7 ± 1 pg/min). In conclusion, stimulation of ETA-R plays an important contributory role to the Ang II–mediated suppression of the activation of renal mechanosensory nerves in conditions of low-sodium diet. (Hypertension. 2007;49:141-147.)

Key Words: ETA-receptors ■ AT1-receptors ■ afferent renal nerves ■ PGE2 ■ substance P ■ BQ123 ■ losartan

The majority of the afferent renal nerves are located in the renal pelvic wall.1-3 Activation of these nerves by increases in renal pelvic pressure results in increases in afferent renal nerve activity (ARNA) leading to reflex decreases in efferent renal sympathetic nerve activity and diuresis and natriuresis, that is, a renorenal reflex response.4

Among the various mechanisms involved in the activation of the renal pelvic wall is induction of cyclooxygenase-2 resulting in increased renal pelvic synthesis of PGE2.5,6 PGE2 leads to activation of the cAMP–protein kinase A transduction pathway and a release of the neuroepitope substance P, which activates the afferent renal nerves by stimulating neurokinin-1 receptors in the pelvic renal area.1,7,8

The responsiveness of the afferent renal nerves is enhanced by high-sodium diet and suppressed by low-sodium diet because of an interaction between prostaglandin (PG) E2 (PGE2) and angiotensin (Ang) II at the peripheral renal sensory nerve endings.7,9,10 In conditions of high-sodium diet, characterized by low endogenous Ang II,11 there is little or no inhibition of the PGE2–mediated activation of adenylyl cyclase. Conversely, in conditions of low-sodium diet, high endogenous Ang II activity reduces the PGE2–mediated activation of adenylyl cyclase via a pertussis–toxin (PTX)–sensitive mechanism leading to an impairment of the renorenal reflexes.

Endothelin (ET)-1, a known modulator of both nociceptors12,13 and baroreceptors,14,15 plays a powerful modulatory role in the activation of renal pelvic mechanosensory nerves.16 ET-1 exerts its effects by activating 2 G protein–coupled receptors, ETA and ETB receptors (Rs).17 Interestingly, the nature of effect of ET-1 on the renorenal reflexes depends on dietary sodium intake. Whereas activation of ETB-R contributes to the enhanced responsiveness of the renal mechanosensory nerves in high-sodium diet, activation of ETA-R contributes to the impaired responsiveness of the renal mechanosensory nerves in low-sodium diet.16

The similar effects of the Ang type I receptor (AT1-R) antagonist losartan and the ETA-R antagonist BQ12318 to enhance the ARNA responses to increased renal pelvic pressure in vivo and the PGE2–mediated release of substance

Received July 6, 2006; first decision July 22, 2006; revision accepted October 2, 2006.
From the Departments of Internal Medicine and Pharmacology, Department of Veterans Affairs Medical Center and University of Iowa Carver College of Medicine, Iowa City.
Correspondence to Ulla C. Kopp, Department of Internal Medicine, VA Medical Center, Building 3, Room 226, Highway 6W, Iowa City, IA 52246.
E-mail ulla-kopp@uiowa.edu
© 2006 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000249634.46212.7b

141
P from isolated renal pelvices in vitro in rats fed low-sodium diet\(^9,16\) suggests an interaction between Ang II and ET-1 in the activation of renal mechanosensory nerves at the peripheral renal nerve endings. There is considerable evidence for such an interaction in the cardiovascular system. Ang II increases the expression of prepro-ET-1 mRNA in various tissues, including the kidney and urinary bladder\(^19\): ET-1 such an interaction in the cardiovascular system. Ang IIeral renal nerve endings. There is considerable evidence for responsiveness of renal mechanosensory nerves in rats on produced by the AT1-R and ETA-R antagonists of the vascular and renal tissue ET-1 levels, which are reduced by an AT1-R antagonist and an ETA-R antagonist,\(^20,22,24–27\) suggesting an important role for stimulation of ETA-R in the cardiovascular responses to Ang II.

AT1-R and ETA-R are widely distributed in the central nervous system associated with cardiovascular control, including the sensory nerves in dorsal root ganglia.\(^28–30\) Although there is currently little evidence for ETA-R in the renal pelvic wall, AT1-Rs have been localized to the renal pelvic wall.\(^31\) Also, we have demonstrated the presence of both Ang II and ET-1 in the renal pelvic wall.\(^10,16\) Because of the demonstrated interaction between Ang II and ET-1 in the cardiovascular system\(^20,22,24–27\) and the similar enhancements produced by the AT1-R and ETA-R antagonists of the responsiveness of renal mechanosensory nerves in rats on low-sodium diet,\(^9,16\) we hypothesized that activation of ETA-R contributes to the Ang II–induced suppression of the renorenal reflexes. We tested this idea by comparing the effects of losartan and BQ123 alone and in combination on the activation of renal mechanosensory nerves in vivo and in vitro in rats fed low-sodium diet. We also examined whether BQ123 reduced the Ang II–induced impairment of the responsiveness of renal mechanosensory nerves in rats fed high-sodium diet.

### Methods

The experimental protocols were approved by the Institutional Animal Care and Use Committee and conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was performed on male Sprague-Dawley rats weighing 199 to 410 g (mean: 286.2 ± 5 g). Two weeks before the study, rats were placed on either sodium (Na\(^+\))-deficient pellets (ICN, Na\(^+\) = 1.6 meq/kg per kilogram) with tap water drinking fluid (low-sodium diet; n = 40) or normal Na\(^+\) pellets (Teklad, Na\(^+\) = 163 meq/kg per kilogram) with 0.9% NaCl drinking fluid (high-sodium diet; n = 20). Urinary sodium excretion averages 0.2 ± 0 and 11 ± 1 mmol/L/24 hours on low- and high-sodium diet, respectively.\(^9,32\)

### In Vivo Studies

Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg IP, Abbott Laboratories) and maintained with an infusion of pentobarbital sodium (0.04 mmol kg\(^{-1}\) hr\(^{-1}\) IV 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 described previously in detail.\(^1,2,6,7,9,10\) In brief, after anesthesia, renal pelvices dissected from the kidneys were placed in wells containing 400 µL of HEPES buffer (pH 7.4), 37°C, containing indomethacin (0.14 mmol/L) to minimize the influence of endogenous PGE\(_2\) on substance P release. Each well contained the pelvic wall from 1 kidney.

The renal pelvic walls were allowed to equilibrate for 130 minutes. The incubation medium was refreshed with fresh HEPES every 10 minutes for the first 120 minutes and every 5 minutes thereafter. The incubation medium was stored at –80°C for later analysis of substance P. The experimental protocol consisted of four 5-minute control periods, one 5-minute experimental period, and four 5-minute recovery periods. PGE\(_2\) was added to the incubation bath to both the ipsilateral and contralateral pelvices during the experimental period.

The in vitro study was divided into 2 groups, groups III and IV. In group III, we compared the effects of losartan or BQ123 alone on the PGE\(_2\)-mediated release of substance P from the ipsilateral pelvis with that produced by the combination of the 2 agents from the contralateral pelvis. The pelvices were derived from rats fed low-sodium diet. In group IV, we compared the effect of Ang II alone with that
produced by the combination of Ang II and BQ123 on the PGE$_2$-mediated substance P release from rats fed high-sodium diet.

**Group III: Effects of an AT1-R and ETA-R Antagonist Alone and in Combination on PGE$_2$-Mediated Release of Substance P From Low-Sodium–Diet Rats**

Three groups of rats were studied. In the first group (n=6), the ipsilateral renal pelvis was incubated in buffer only and the contralateral renal pelvis in buffer containing losartan (0.44 mmol/L) throughout the control, experimental, and recovery periods. In the second and third groups (n=8), the ipsilateral pelvis was incubated in buffer containing losartan (n=8) or BQ123 (1 $\mu$mol/L)\(^6\) (n=8) and the contralateral pelvis in buffer containing a mixture of losartan and BQ123. During the experimental period, the ipsilateral and contralateral pelvices were exposed to PGE$_2$ at 0.14 $\mu$mol/L, a subthreshold concentration for substance P release in low-sodium–diet rats.\(^9\)

**Group IV: Effects of Ang II Alone and in Combination With an ETA-R Antagonist on PGE$_2$-Mediated Release of Substance P From High-Sodium Diet Rats**

Two groups were studied. In the first group (n=6), the ipsilateral pelvis was incubated in buffer only and the contralateral pelvis in buffer containing Ang II (15 nM). In the second group (n=12), the ipsilateral pelvis was incubated in buffer containing Ang II and the contralateral pelvis in buffer containing a mixture of Ang II and BQ123 (1 $\mu$mol/L). During the experimental period, the ipsilateral and contralateral pelvices were exposed to PGE$_2$ at 0.03 $\mu$mol/L, a concentration known to increase substance P release in high-sodium–diet rats.\(^9\)

**Drugs**

Losartan was supplied by Merck. Substance P antibody (IHC 7451) was acquired from Penninsula Laboratories and PGE$_2$ from Cayman Chemicals. All of the other agents were from Sigma Chemicals. Indomethacin was dissolved together with Na$_2$CO$_3$ (2:1 weight ratio) in HEPES buffer and all of the other agents in 0.15 mol/L NaCl or incubation buffer.

**Analytical Procedures**

Substance P in the incubation medium was measured by ELISA, as described previously in detail.\(^1,2,6,7,9,10\)

**Statistical Analysis**

Friedman 2-way ANOVA and shortcut ANOVA were used to determine the effects of the various treatments on ARNA and the release of substance P within each rat/pelvis. Kruskal–Wallis 1-way ANOVA and multiple comparisons between treatments were used to determine possible differences between groups in vivo, and the Wilcoxon matched-pairs signed-rank test was used to compare the increases in substance P release in the ipsilateral and contralateral renal pelvices in vitro. A significance level of 5% was chosen. Data in text and Figure 1 are expressed as mean±SE.\(^3,3,34\)

**Results**

**In Vivo Studies**

**Group I: Effects of an AT1-R and ETA-R Antagonist Alone and in Combination on the ARNA Responses to Increased Renal Pelvic Pressure in Rats Fed Low-Sodium Diet**

We tested the hypothesis that Ang II and ET-1 suppress the responsiveness of the renal mecanosensory nerves by a common pathway. In the presence of renal pelvic perfusion with vehicle, increasing renal pelvic 7.5 mm Hg resulted in similar small increases in ARNA in the 2 groups, which only reached statistical significance in 1 group (Figure 1A). Renal pelvic perfusion with losartan (Figure 1A) or BQ123 (Figure 1B) resulted in similar enhancements of the ARNA responses to increased renal pelvic pressure. Subsequent perfusion of the renal pelvis with a mixture of losartan and BQ123 did not produce any further enhancements of the ARNA responses in either group. Mean arterial pressure (111±4 and 119±2 mm Hg) remained unchanged throughout the experiment.

**Group II: Effects of Ang II Alone and in Combination With an ETA-R Antagonist on the ARNA Responses to Increased Renal Pelvic Pressure in Rats Fed High-Sodium Diet**

To examine whether activation of ETA-R contributes to the Ang II–induced impairment of the activation of renal mechanosensory nerves, we examined whether BQ123 blocked the Ang II–mediated suppression of the ARNA response to increased renal pelvic pressure. In the presence of renal pelvic perfusion with vehicle, increasing renal pelvic 7.5 mm Hg resulted in marked increase in ARNA (Figure 2) that was significantly greater than that produced in the low-sodium–diet rats (P<0.01; Figure 1). Renal pelvic perfusion with
Ang II blocked the ARNA response to increasing renal pelvic pressure. Adding BQ123 to the renal pelvic perfusate containing Ang II restored the ARNA response toward that produced in the presence of vehicle. Mean arterial pressure (117±3 mm Hg) remained unaltered throughout the experiment.

In Vitro Studies
Because losartan, BQ123, and Ang II were administered directly into the renal pelvis and produced no effects on mean arterial pressure, the results in groups I and II suggested that the interaction between ET-1 and Ang II may occur at the peripheral renal sensory nerves. We examined this issue by studying the effects of losartan and BQ123 on the PGE₂-mediated release of substance P using an isolated renal pelvic wall preparation.

Group III: Effects of an AT₁-R and an ETA-R Antagonist Alone and in Combination on the PGE₂-Mediated Release of Substance P From Low-Sodium–Diet Rats
Adding 0.14 μmol/L of PGE₂ to the incubation bath containing vehicle-treated pelvises from low-sodium–diet rats failed to produce a release of substance P (Figure 3A). However, in the presence of losartan, the same concentration of PGE₂ produced a significant increase in substance P release. Similar increases in PGE₂-mediated release of substance P were obtained from renal pelvises incubated in losartan and losartan+BQ123 (Figure 3B), the increases being significantly greater than that produced in the presence of vehicle (Figure 3A; P<0.01). Likewise, PGE₂ produced similar increases in substance P release from pelvises incubated in BQ123 and BQ123+losartan (Figure 3C), the increases being of a similar magnitude as those produced by losartan and losartan+BQ123 (Figure 3B). Concurrent studies in low-sodium–diet rats showed that the PGE₂-mediated increase in substance P release in the presence of BQ123 was greater than that produced in the presence of vehicle.\textsuperscript{16}

Group IV: Effects of Ang II Alone and in Combination With an ETA-R Antagonist on PGE₂-Mediated Release of Substance P From High-Sodium–Diet Rats
PGE₂ at 0.03 μmol/L produced a marked release of substance P from vehicle-treated pelvises (Figure 4A). However, the same concentration of PGE₂ failed to increase substance P release from the contralateral pelvis incubated with Ang II. Adding BQ123 to the incubation bath containing Ang II restored the PGE₂-mediated release (Figure 4B) toward that seen in vehicle-treated pelvises (Figure 4A). Importantly, concurrent studies in high-sodium–diet rats show that BQ123, per se, that is, in the absence of exogenous Ang II,
Taken together, these studies demonstrated the effect of ETA-R antagonists on the responsiveness of renal mechanosensory nerves. This effect was observed in conditions of high-sodium dietary intake, AT1-R or ETA-R agonists having no effects on the responsiveness of renal sensory nerves.9,16

The current studies showed that renal pelvic perfusion with losartan, BQ123, or a combination of losartan+BQ123 produced similar enhancements of the ARNA responses to increased renal pelvic pressure in rats fed low-sodium diet. Concurrent studies in rats fed low-sodium diet16 showed that repeated increases in renal pelvic pressure in the presence of BQ123 alone result in reproducible increases in ARNA. Thus, the lack of a further enhancement of the ARNA responses to increased renal pressure in the presence of the combination of losartan and BQ123 compared with that produced by either agent alone is not because of a time-related decreased responsiveness of the renal pelvic sensory nerves.

One may postulate that the lack of further enhancement of the ARNA responses in the presence of BQ123+losartan compared with that produced by either agent alone may be related to the activation of renal mechanosensory nerves being maximized by losartan and/or the concentration of BQ123 not being sufficiently high. However, the following data would argue against this reasoning: (1) our previous studies in vehicle-treated low-sodium–diet rats have shown that graded increases in renal pelvic pressure result in graded increases in ARNA that do not reach a maximum within the range tested.9 (2) BQ123 was administered at a concentration shown to produce maximum inhibition of ET-1 binding in ETA–R–enriched preparations.18 (3) The ARNA responses to increased renal pelvic pressure in low-sodium–diet rats treated with BQ123 are not different from those in vehicle-treated high-sodium–diet rats (P > 0.10) in which activation of ETA-R does not contribute to the activation of renal mechanosensory nerves.16 It is unlikely that the variability in the ARNA responses in the 2 groups in the current study obscured a true difference between the 2 groups, because our previous studies in similarly treated rats showed similar ARNA responses (P = 0.07) in low-sodium–diet rats treated with BQ123 (3 groups; total N = 27) and high-sodium–diet vehicle-treated rats (3 groups; N = 24).16

To further support the hypothesis that activation of ETA-R contributes to the Ang II–mediated suppression of the activation of renal mechanosensitive nerve fibers, we examined whether BQ123 would alter the ARNA response to increased renal pelvic pressure in Ang II–treated kidneys. Studies were performed in high-sodium–diet rats to minimize the influence of endogenous Ang II.11 In agreement with our previous studies,9 renal pelvic perfusion with Ang II suppressed the ARNA response to increased renal pelvic pressure. Adding BQ123 to the renal pelvic perfusate containing Ang II restored the ARNA response toward that produced in the presence of vehicle. Studies in bovine cerebellum and rat cardiomyocytes18,20 showing that BQ123 at 10 μmol/L does not inhibit the specific binding of Ang II or Ang II–induced protein synthesis suggest that BQ123 at the concentrations used in the current studies does not enhance the responsiveness of the renal mechanosensory nerves by blocking AT1-R.

Although not studied in this study, it is likely that the enhancement of the activation of renal mechanosensory nerves produced by BQ123 in low-sodium–diet rats and by the addition of BQ123 to Ang II–treated pelvises in high-sodium–diet rats is related to activation of ETB–R.
concurrent studies showed that the ETB-R antagonist BQ788 blocked the BQ123-induced enhancement of the PGE2-mediated release of substance P and ARNA response to increased renal pelvic pressure in low-sodium–diet rats.16

To explore whether the interaction between Ang II and ET-1 involved mechanisms prior or distal to the increased renal pelvic PGE2 synthesis produced by the stretching the pelvic wall, we used an isolated renal pelvic wall preparation to compare the release of substance P produced by exogenous administration of PGE2 to the incubation bath in the presence of AT1-R and ETA-R antagonists alone and in combination. In agreement with our previous studies,9,16 acute administration of losartan and BQ123 alone enhanced the PGE2-mediated release of substance P from pelvises derived from low-sodium–diet rats. Administration of losartan plus BQ123 did not produce any further enhancement of the PGE2-mediated substance P release beyond that produced by either agent alone. Subsequent studies in renal pelvises derived from high-sodium–diet rats showed that Ang II suppressed the substance P release produced by PGE2, in agreement with our previous studies.10 The current studies further showed that the Ang II–mediated suppression of the PGE2-mediated substance P release was markedly abrogated by the simultaneous presence of BQ123.

Our previous studies showed that Ang II suppresses the activation of renal mechanosensory nerves by inhibiting the PGE2-mediated activation of adenylyl cyclase by a PTX-sensitive mechanism.10 Although ET-1–mediated activation of ETA-R has been shown to both increase and decrease cAMP accumulation,36 there are numerous reports providing evidence for ET-1 inhibiting cAMP accumulation by a PTX-sensitive mechanism. In inner medullary collecting ducts, ET-1 inhibits an arginine vasopressin-induced increase in water permeability by a PTX-sensitive mechanism.37 Likewise, in gall bladder epithelial cells, ET-1 reduces cAMP accumulation by a PTX-sensitive mechanism.38 ET-1–mediated activation of ETA-R inhibits voltage-sensitive Ca++ channels in cultured cerebellar neurons and the isooproterenol-induced increases in L-type Ca++ current in ventricular myocytes,40,41 the latter involving a PTX-sensitive mechanism.

The mechanisms involved in the interaction between AT1-R and ETA-R in renal mechanosensory activation are currently unknown. Dietary sodium does not alter ET-1 concentration in renal pelvic tissue16 suggesting that the interaction between AT1-R and ETA-R does not involve increased ET-1. Although it is presently not known whether low-sodium diet alters ETA-R density in renal pelvic tissue, the activation of ETA-R after acute administration of Ang II in high-sodium–diet rats may argue against increased density of ETA-R being the mechanism or only mechanism involved in the interaction between the 2 receptors. Rather, we hypothesize that the interaction between the receptors may be the result of a cross-talk between AT1-R and ETA-R, which may involve heterodimerization of the 2 receptors and/or Ang II–induced activation of AT1-R activating a mechanism(s), which, in turn, would result in activation of ETA-R. Numerous reports from in vitro studies demonstrate various G protein–coupled receptors forming heterodimers, including ETA-R and ETB-R, and AT1-R and bradykinin-2R.42,43

Taken together, our in vivo and in vitro studies suggest that stimulation of ETA-R contributes to the Ang II–mediated suppression of renal mechanosensory nerves by a mechanism distal to renal pelvic PGE2 synthesis. In view of our and other investigators’ studies,11,37–41 we hypothesize that Ang II activates AT1-R, resulting in increased activation of ETA-R, which, in turn, suppresses the PGE2-mediated release of substance P and activation of renal mechanosensory nerves by a PTX-sensitive mechanism.

Perspectives
Selective afferent renal denervation results in salt-sensitive hypertension, demonstrating the physiological relevance of the renorenal reflexes in conditions of high-sodium dietary intake.32 Suppression of renorenal reflexes in conditions of low-sodium dietary intake is an appropriate response contributing to the preservation of sodium and water. The current studies showing that activation of ETA-R contributes importantly to the Ang II–induced suppression of the responsiveness of renal mechanosensory nerves suggest that ET-1 stimulation of ETA-R plays an important role in the maintenance of sodium and water balance.

Sources of Funding
This work was supported by grants from the Department of Veterans Affairs, the National Institutes of Health, National Heart, Lung, and Blood Institute (RO1 HL66068), and Specialized Center of Research grant HL55006.

Disclosures
None.

References
Interaction Between Endothelin and Angiotensin


Activation of Endothelin-A Receptors Contributes to Angiotensin-Induced Suppression of Renal Sensory Nerve Activation
Ulla C. Kopp, Michael Z. Cicha and Lori A. Smith

Hypertension. 2007;49:141-147; originally published online October 23, 2006; doi: 10.1161/01.HYP.0000249634.46212.7b
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/49/1/141

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/