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

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Running Head: Endothelin modulates afferent renal nerve activity

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Activation of renal mecanosensory nerves is enhanced by high and suppressed by low sodium diet. Afferent renal denervation results in salt sensitive hypertension suggesting that activation of the afferent renal nerves contributes to water and sodium balance. Another model of salt sensitive hypertension is the endothelin B receptor (ETB-R) deficient rat. ET and its receptors are present in sensory nerves. Therefore, we examined whether ET-R blockade altered the responsiveness of the renal sensory nerves. In anesthetized rats fed high sodium diet, renal pelvic administration of the ETB-R antagonist BQ788 reduced the afferent renal nerve activity (ARN) response to increasing renal pelvic pressure 7.5 mmHg from 26±3 to 9±3% and the PGE$_2$-mediated renal pelvic release of substance P from 9±1 and 3±1 pg/min. Conversely in rats fed low sodium diet, renal pelvic administration of the ETA-R antagonist BQ123 enhanced the ARN response to increased renal pelvic pressure from 9±2% to 23±6% and the PGE$_2$-mediated renal pelvic release of substance P from 0±0 to 6±1 pg/min. Adding the ETA-R antagonist to ETB-R-blocked renal pelvises restored the responsiveness of renal sensory nerves in high sodium diet rats. Adding the ETB-R antagonist to ETA-R- blocked pelvises suppressed the responsiveness of the renal sensory nerves in low sodium diet rats. Conclusion: Activation of ETB-R and ETA-R contributes to the enhanced and suppressed responsiveness of renal sensory nerves in conditions of high and low sodium intake, respectively. Impaired renorenal reflexes may contribute to the salt sensitive hypertension in the ETB-R deficient rat.

Keywords: ETB receptor, ETA receptor, substance P, PGE$_2$
INTRODUCTION

The majority 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). Activation of this inhibitory reflex contributes together with the cardiovascular reflexes, including the aortic and carotid baroreceptor reflexes, 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 PGE$_2$ (34,36). PGE$_2$ stimulates EP4 receptors on or close to the renal sensory nerves leading to activation of the cAMP - protein kinase A transduction pathway and a Ca-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 diet due to an interaction between PGE$_2$ and angiotensin (ANG) II at the peripheral sensory nerve endings (30, 33). In conditions of low sodium diet, high endogenous ANG II activity reduces the PGE$_2$-mediated activation of adenylyl cyclase via a pertussis toxin (PTX) sensitive mechanism (30) leading to an impairment of the renorenal reflexes. Conversely in conditions of high sodium diet, characterized by low endogenous ANG II (9), there is little or no inhibition of the PGE$_2$-mediated activation of adenylyl cyclase. The increased responsiveness of the afferent renal nerves in conditions of high sodium diet 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 (11, 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 which 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 ANG II when rats are fed high but not when they are fed normal sodium diet (23).

Another model of salt sensitive hypertension is the endothelin B receptor (ETB-R) deficient rat (15,47). Endothelin (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-receptors (R) (51). The responses to ET vary with the cell type/organ. The major vascular effects of ETA-receptor (ETA-R) activation is vasoconstriction. The results of activation of ETB-R are more diverse, including vasoconstriction, vasodilation and diuresis and natriuresis (51). In the kidney, ET-1 is widely distributed, with the highest concentration in inner medulla (51,61). Whereas, ETA-R are predominantly localized to the renal vasculature. ETB-R are found in glomeruli, inner medullary collecting duct cells and the renal pelvic area (69).

The mechanisms involved in the salt sensitive hypertension in the ETB-R deficient rat are not completely understood. A role for ETA-R in the increased arterial pressure has been suggested
by studies showing marked reduction of the hypertension by ETA-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 which is reduced by ETA-R antagonists in a similar fashion as that in ETB-R deficient rats (15, 47).

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

The known modulatory role of ET-1 on nociceptors (6, 25, 26) and baroreceptors (4, 22, 40) taken together with the salt sensitive hypertension in afferent renal denervated rats (31) suggest that an impairment of the renorenal reflexes may contribute to the salt sensitive hypertension in ETB-deficient rats. Therefore, we examined the role of ET-1 on the activation of renal mechanosensory nerves by studying the effects of ETB-R and ETA-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, rats were placed on either sodium (Na⁺)-deficient pellets (ICN, Na⁺=1.6 meq/kg) with tap water drinking fluid (low sodium diet, n=75), normal Na⁺ pellets (Teklad, Na⁺=163 meq/kg) with tap water drinking fluid (normal sodium diet, n=28) or normal Na⁺ 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 i.p. Abbott Laboratories).

Renal and DRG tissue ET-1 concentrations. The renal pelvis, medulla and cortex and DRG (T₉-L₁) 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⁻¹ · hr⁻¹) 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-second intervals, the unit of measure being microvolts per second per 1 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 in 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, we examined the effects of an ETB-R and an ETA-R antagonist on the ARNA responses to increasing renal pelvic pressure in rats fed high, normal and low sodium diet. In Group II we examined whether an ETA-R antagonist blocked the effects produced by an ETB-R antagonist on the ARNA responses in rats fed high sodium diet. We also examined whether an ETB-R antagonist blocked the effects of an ETA-R antagonist on the ARNA responses in rats fed low sodium diet. In Group III, which served as time controls, renal pelvic pressure was increased in the absence of any ET-R 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 IA: Effects of an ETB-R antagonist on the 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 ETB-R antagonist BQ788 (21), 1 μM. Ten minutes later the two control, experimental and recovery periods were repeated.

**Group IB: Effects of an ETA-R antagonist on the 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 in Group IA except the renal pelvis was perfused with the ETA-R antagonist BQ123 (20). Because pilot experiments showed inconsistent effects of BQ123 at 1 μM, the studies were performed using BQ123 at 5 μM.

**Group II: Effects of an ETB-R and an ETA-R antagonist alone and in combination on the ARNA responses to increased renal pelvic pressure.** Rats were fed high or low sodium diet. The
experiment was divided into three parts. During each part, renal pelvic pressure was increased 7.5 mmHg during the experimental period. In rats fed high sodium diet, the renal pelvic perfusate was switched from vehicle to BQ788 (1 μM) at the end of the first part. Twenty minutes later, the control, experimental and recovery periods were repeated. At the end of the second part, the renal pelvic perfusate was switched from BQ788 to a perfusate containing either a mixture of BQ788 (1 μM) and BQ123 (5 μM) (n=8) or remained the same (i.e., BQ788 only) (n=6). Twenty minutes later the control, experimental and recovery periods were repeated once more.

In rats fed low sodium diet, the experimental protocol was similar to that above, except renal pelvis was perfused with BQ123 (5 μM) (n=13) during the second part and BQ123+BQ788 during the third part or with BQ123 only (n=6) during the last two parts of the experiment.

**Group III:** *Increasing renal pelvic pressure in the absence of ET-R antagonists (time controls).* Rats were fed high sodium diet (n=10) or low sodium diet (n=10). The experimental protocol was similar to that in Groups 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 ETB-R and ETA-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 ET-R 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, following anesthesia renal pelvises dissected from the kidneys were placed in wells containing 400 μl HEPES
(25 mM HEPES, 135 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, 3.3 mM d-glucose, 0.1 mM ascorbic acid, 0.1% BSA, 10 μM dl-thiorphan, 1 mM Phe-Ala, 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 PGE₂ on substance P release. Each well contained the pelvic wall from one kidney.

The renal pelvic walls were allowed to equilibrate for 130 minutes. The incubation medium was replaced with fresh HEPES every 10 minutes for the first 120 min and every 5 min thereafter. The incubation medium was placed in 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. PGE₂ was added to the incubation bath to both the ipsilateral and contralateral pelvises.

The in vitro study was divided into two main groups, Groups IV&V. In Group IV, we examined whether incubating the renal pelvic wall with an ETB-R or an ETA-R antagonist altered the PGE₂-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 BQ788 and BQ123 alone with those of the combination of BQ123 and BQ788 on the PGE₂-mediated release of substance P in rats fed high and low sodium diet, respectively.

The various concentrations of PGE₂ used in Groups IV and V represent those that are subthreshold for substance P release in rats fed high, normal and low sodium diet, 0.014, 0.03 and 0.14 μM, respectively and those that are required to produce an increase in substance P release in rats fed high and normal sodium diet, 0.03 and 0.14 μM, respectively (32).

**Group IVA: Effects of an ETB-R antagonist on PGE₂-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 BQ788, 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 PGE$_2$ at 0.14 μM in the normal sodium diet group (N=8).

**Group IVB: Effects of an ETA-R antagonist on PGE$_2$-mediated release of substance P.** Rats were fed high, normal or low sodium diet. The first group (n=6) was fed high, the second (n=8) and third groups (n=8) normal and the fourth group (n=15) low sodium diet. The experimental protocol was similar to that described above except the contralateral pelvis was incubated in HEPES/indomethacin buffer containing BQ123 throughout the experiment. Because our initial experiments in the low sodium diet group showed similar effects produced by 1 and 5 μM BQ123, all subsequent studies used BQ123 at 1 μM. During the experimental period, the ipsilateral and contralateral pelvises were exposed to PGE$_2$, 0.014 μM (n=3) and 0.03 μM (n=6) in rats fed high sodium diet and PGE$_2$ at 0.14 μM in rats fed low sodium diet (n=15). Pelvises from rats fed 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 ETB-R and an ETA-R antagonist alone and in combination on PGE$_2$-mediated release of substance P.** Rats were fed high sodium diet (n=14) or low sodium diet (n=13). Ipsilateral pelvises from high and low sodium diet rats were incubated HEPES/indomethacin buffer containing BQ788, 1 μM, and BQ123, 1 μM, respectively. The contralateral pelvises from either group were incubated in HEPES/indomethacin buffer containing BQ788+BQ123, both at 1 μM. During the experimental period, PGE$_2$ at 0.03 and 0.14 μM was added to both pelvises from the high and low sodium diet rats, respectively.

*Drugs.*
Substance P antibody (IHC 7451) was acquired from Penninsula Laboratories (San Carlos, CA) and PGE\textsubscript{2} from Cayman Chemicals (Ann Arbor, MI). All other agents were from Sigma Chemicals (St. Louis, MO) unless otherwise stated. Indomethacin was dissolved together with Na\textsubscript{2}CO\textsubscript{3} (2:1 weight ratio) in HEPES buffer and all other agents in incubation buffer (\textit{in vitro} studies) or 0.15 M NaCl (\textit{in vivo} studies).

\textit{Analytical Procedures}

Substance P in the incubation medium was measured by ELISA, as previously described in detail (30,32,35,36).

Tissue ET-1 concentrations were determined using an ET-1 QuantiGlo chemiluminescent immunoassay kit (R&D Systems, Minneapolis, MN). Renal pelvic and DRG tissues were homogenized in 1 ml, renal cortical tissue in 5 ml and papillary tissue in 2.5 ml of 1 M acetic acid containing pepstatin A, 10 \mu g/ml. The homogenates were then heated to 100°C for 10 min, and centrifuged at 15,800g for 30 min at 4°C. ET-1 concentrations were determined in the supernatants (52). Tissue protein concentration was determined using the Bio-Rad assay kit.

\textit{Statistical Analysis}

\textit{In vivo}, ARNA, systemic hemodynamics and renal excretion were measured and averaged over each period. Friedman 2-way analysis of variance and shortcut analysis of variance were used to determine the effects of the various treatments on the ARNA and natriuretic responses within each rat. \textit{In vitro}, the release of substance P during the experimental period was compared with that during the control and recovery periods using Friedman 2-way analysis of variance and shortcut analysis of variance. The Wilcoxon matched-pairs signed-rank test was used to compare the value of ipsilateral and contralateral renal pelvic release of substance P during the experimental period with the average of the control and recovery periods. A significance level of 5% was chosen. Data in text
and figures are expressed as means ± SE (53, 57).

RESULTS

Renal and DRG tissue ET-1 concentrations. As seen in Fig. 1, ET-1 is present in renal pelvic tissue at a concentration about 15-fold lower than that in papillary tissue but ten-fold higher than that in cortical tissue. ET-1 was also present in tissue from DRG at T₉-L₁. 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 ETB-R antagonist on the ARNA responses to increased renal pelvic pressure. The presence of ET-1 in renal pelvic tissue and DRG (T₉-L₁)(Fig. 1) and ETB-R in renal pelvic wall (69) suggested that activation of ETB-R may modulate renal sensory nerves. As seen in Fig. 2, renal pelvic perfusion with the ETB-R antagonist BQ788 suppressed the ARNA responses to increasing renal pelvic pressure in rats fed high but not in rats fed normal sodium diet. The increases in contralateral urinary sodium excretion produced by increased renal pelvic pressure reached statistical significance in response to increasing renal pelvic pressure 7.5 mmHg during vehicle perfusion in the high sodium diet rats, urinary sodium excretion being increased from 1.0±0.2 to 1.3±0.3 μmol·min⁻¹·g⁻¹ (P<0.01). Mean arterial pressure remained unaltered throughout the experiments, being 114±3 and 108±3 mmHg in rats fed high and normal sodium diet, respectively.

Group IB: Effects of an ETA-R antagonist on the ARNA responses to increased renal pelvic pressure in rats. Because of the well-documented effects of ETA-R activation on nociceptors, we speculated that an ETA-R antagonist may 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 seen in Fig. 3, renal pelvic perfusion with the ETA-R antagonist BQ123 produced a significant
enhancement of the ARNA responses to increasing renal pelvic pressure 2.5 and 7.5 mmHg in rats fed low but not in rats fed normal sodium diet. Mean arterial pressure remained unaltered throughout the experiments in both groups of rats, being 114±2 115±6 mmHg in rats fed low and normal sodium diet, respectively.

**Group II: Effects of an ETB-R and an ETA-R antagonist alone and in combination on the ARNA responses to increased renal pelvic pressure.** The results in Group IB together with those showing that an ETA-R antagonist reduced arterial pressure in ETB-R deficient rats fed high sodium diet (15, 47) suggested that the suppressed ARNA response to increased renal pelvic pressure in the presence of ETB-R blockade in high sodium diet rats maybe due to activation of ETA-R. Therefore in rats fed high sodium diet, we examined whether an ETA-R receptor antagonist would restore the BQ788-induced reduction of the ARNA responses to increased renal pelvic pressure towards control responses. As seen in Fig. 4 and similar to our previous studies (Fig. 2), renal pelvic perfusion with BQ788 produced a marked reduction of the ARNA response to increasing renal pelvic pressure 7.5 mmHg. Adding BQ123 to the renal pelvic perfusate containing BQ788 restored the ARNA response towards its response in the presence of vehicle. Continued renal pelvic perfusion with BQ788 without the addition of BQ123 resulted in a maintained suppression of the ARNA responses to increased renal pelvic pressure (Table 1). Mean arterial pressure, 118±3 and 123±5 in the two groups remained unaltered throughout the experiment.

Because these studies suggested that the impaired responsiveness of the renal mechanosensory nerves following ETB-R blockade was due to ET-1 activating ETA-R (Fig. 4), we examined whether a similar interaction between the two ET-R occurred in conditions of low dietary sodium intake. As seen in Fig. 4 and similar to our previous studies in low sodium diet rats (Fig. 3), renal pelvic perfusion with BQ123 enhanced the ARNA response to increasing renal pelvic pressure
7.5 mmHg. Adding BQ788 to the renal pelvic perfusate containing BQ123 suppressed the ARNA response towards that seen during vehicle perfusion. Continued renal pelvic perfusion with BQ123 without the addition of BQ788 resulted in similar enhanced ARNA responses to increased renal pelvic pressure (Table 1). Mean arterial pressure, 110±5 and 113±2 mmHg, remained unaltered throughout the experiment in the two groups.

**Group III: Increasing renal pelvic pressure in the absence of ET-R antagonists (time controls).** In rats fed high sodium diet, increasing renal pelvic pressure 7.5 mmHg three times in the presence of vehicle resulted in reproducible increases in ARNA (Table 2) and contralateral urinary sodium excretion, 23±9% from 1.0±0.3 μmol·min⁻¹·g⁻¹, 25±6% from 1.3±0.3 μmol·min⁻¹·g⁻¹ and 25±13% from 1.6±0.4 μmol·min⁻¹·g⁻¹ (all P<0.05). Also in rats fed low sodium diet, increasing renal pelvic pressure 7.5 mmHg three times in the presence of vehicle resulted in reproducible but somewhat smaller ARNA responses (Table 2). The increases in contralateral urinary sodium excretion produced by increased renal pelvic pressure failed to reach statistical significance, 0±5% from 0.7±0.5 μmol·min⁻¹·g⁻¹, 8±4% from 0.8 μmol·min⁻¹·g⁻¹ and 8±7% from 1.3 μmol·min⁻¹·g⁻¹. Mean arterial pressure, 116±5 109±2 mmHg, remained unaltered in the two groups.

**In Vitro**

Among the mechanisms involved in the activation of the afferent renal nerves following an increase in renal pelvic pressure 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 BQ788 and BQ123 on the PGE₂-mediated release of substance P.

**Group IVA: Effects of an ETB-R antagonist on PGE₂-mediated release of substance P.** As
seen in Fig. 5 and similar to our previous studies, substance P was released into the incubation bath by PGE₂ at a five-fold lower concentration in rats fed high (0.03 μM) than normal (0.014 μM) sodium diet (32). Adding BQ788 to the bath markedly suppressed the PGE₂-mediated substance P release from pelvises derived from high sodium diet fed rats but had no effect on the substance P release from pelvises derived from normal sodium diet fed rats.

**Group IVB: Effects of an ETA-R antagonist on PGE₂-mediated release of substance P.** As seen in Fig. 6 and in agreement with our previous studies (32), PGE₂ at a concentration that increased substance P release from pelvises derived from normal sodium diet fed rats failed to increase substance P release from pelvises derived low sodium diet fed rats. Adding BQ123 at 5 or 1 μM to the bath containing the pelvises of low sodium diet rats enhanced the PGE₂-mediated substance P release to the same extent, so the data have been pooled. BQ123 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 normal sodium diet rats showed that BQ123 produced a small enhancement of the substance P release produced by a subthreshold concentration of PGE₂ (0.03 μM), the magnitude of the substance P release being less than that produced from pelvises derived from low sodium diet rats treated with BQ123 (P<0.05). On the other hand in rats fed high sodium diet, BQ123 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 high sodium diet rats); vehicle treated pelvises: from 8.8±5.9 to 11.4±7.7 and BQ123-treated pelvises: from 6.3±3.0 to 6.3±2.4 (N=3).

**Group V: Effects of an ETB-R and an ETA-R antagonist alone and in combination on the 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 BQ788 (Fig. 5) and BQ788+BQ123 (Fig. 7), respectively, showed that the BQ788-mediated suppression of the PGE₂-
mediated release of substance P was prevented by incubating the pelvises in a combination of BQ788+BQ123. Likewise, comparing the PGE₂-mediated release of substance P from ipsilateral and contralateral pelvises from low sodium diet rats incubated with BQ123 (Fig. 5) and BQ123+BQ788 (Fig. 7), respectively, showed that the BQ123-mediated enhancement of the PGE₂-mediated release of substance P was not observed when the pelvises were incubated in a combination of BQ123+BQ788.

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 ETB-R antagonist suppresses the PGE₂-mediated release of substance P and the ARNA response to increased renal pelvic pressure in conditions of high sodium dietary intake. Conversely in conditions of low dietary sodium intake, renal pelvic administration of an ETA-R antagonist enhances the PGE₂-mediated release of substance P and the ARNA response to increased renal pelvic pressure. In conditions of normal dietary sodium intake, the activation of renal mechanosensory nerves is slightly enhanced by an ETA-R antagonist but not affected by an ETB-R antagonist. Taken 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 ETA-R are expressed in sensory nerve bodies in lumbar DRG (16), ETB-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 ETA-R located on the sensory nerve fibers and ETB-R on peripheral glia cells surrounding sensory nerve fibers. These studies taken together with the presence of ET-1 in renal pelvic tissue and DRG (T₉-L₁) (current
studies) and ETB-R in the renal pelvic wall (69) may 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 ET3, the latter having higher affinity for ETB-R than ETA-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 ETA-R (40).

Renal mechanosensory nerve activation: Role of ET-mediated activation of ETB-R and ETA-R. The threshold pressure for activation of renal pelvic mechanosensory nerves being less than 2.5 mmHg in rats fed high sodium diet (32) suggest that the renal mechanosensory nerves are tonically active in conditions of high dietary sodium intake. This idea is supported by studies showing that unilateral renal denervation produces a renorenal reflex increase in contralateral ERSNA and decrease in contralateral urinary sodium excretion in volume expanded rats (10). Also, selective bilateral afferent renal denervation results in increased arterial pressure in rats fed high but not in rats fed normal sodium diet (31).

There is considerable evidence for ET playing an important role in the maintenance of water and sodium balance. The ETB-R 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 ETB-R 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 ETB-R (24,51,65).
The present studies in high sodium diet rats show that renal pelvic administration of the ETB-R antagonist BQ788 suppressed the ARNA responses to increased renal pelvic pressure. Importantly, the ETB-R antagonist had no effect on the responsiveness of the renal mechanosensory nerves in rats fed 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). Taken together, these studies may suggest that an impaired responsiveness of the renal mechanosensory nerves in the ETB-R deficient rat contributes to the salt sensitive hypertension.

In conditions of low dietary sodium intake, when endogenous ANG II is increased (9), the activation threshold 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). Non-neural cardiovascular and renal responses to ANG II are reduced by an ETA-R antagonist (7,50), suggesting that ET-1 by activating ETA-R 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 ETA-R antagonist may enhance the responsiveness of the renal mechanosensory nerves in a similar fashion as an AT-1 receptor antagonist (32). Our findings supported our hypothesis. In conditions of low sodium diet, renal pelvic administration of the ETA-R antagonist BQ123 enhanced the ARNA responses to increased renal pelvic pressure. In normal sodium diet rats, the ETA-R antagonist had no significant effect on the ARNA responses to increasing in renal pelvic pressure below or above the threshold for activation of renal mechanosensory nerves, i.e. 2.5 and 7.5 mmHg. Taken together, our findings suggest that ET-1 via activation of ETA-R contributes to the suppression of the
responsiveness of renal pelvic mechanosensory nerves in conditions of low dietary sodium 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 ET-R antagonists would involve mechanisms before and/or after the increased renal pelvic PGE$_2$ synthesis produced by the stretching the pelvic wall. By design, this experimental model also excludes systemic and central mechanisms that may modulate the activation of renal sensory nerves. Our data showing that the PGE$_2$-mediated release of substance P was suppressed by acute administration of an ETB-R antagonist to renal pelvises derived from high sodium diet rats and enhanced by acute administration of an ETA-R antagonist to renal pelvises derived from low sodium diet rats suggest that ET-1 modulates the PGE$_2$-mediated activation of renal sensory nerves by a mechanism(s) at the peripheral sensory nerve terminals.

BQ123 and BQ788 are selective ETA-R and ETB-R antagonists with no agonist activity at the concentrations used (20,21). To examine whether the effects produced by the two ET-R antagonists were specific to each antagonist and the dietary sodium intake, we examined the effects of BQ788 and BQ123 in rats fed various sodium diets. Whereas, BQ788 suppressed the responsiveness of the renal sensory nerves in rats fed high sodium diet both in vivo and in vitro, BQ788 had no effect in rats fed normal sodium diet. Conversely, BQ123 enhanced the responsiveness of the renal mechanosensory nerves in rats fed low and normal sodium diet, the enhancement being greater in rats fed low than normal sodium diet. Importantly, BQ123 neither enhanced nor suppressed the PGE$_2$-mediated substance P release in rats fed high sodium diet. Taken together, these findings suggest that the effects produced by BQ788 and BQ123 were specific to the ETB-R and ETA-R, respectively, and to the various dietary sodium intakes.

*Activation of renal mechanosensory nerves: Interaction between ETB-R and ETA-R. Studies*
in ETB-R deficient rats have shown that activation of ETA-R contributes to the salt sensitive hypertension (15,47). Likewise, our in vivo and in vitro studies show that the impaired responsiveness of the renal mechanosensory nerves following renal pelvic administration of the ETB-R antagonist was due to activation of ETA-R, the activation of ETA-R being revealed by blocking the ETB-R. The enhanced activation of ETA-R in the absence of functioning ETB-R has been suggested to involve increased plasma concentrations of ET-1, due to the ETB-R 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-R antagonist following ETB-R blockade in vivo, it is unlikely that this mechanism would explain the enhanced effect of the ETA-R 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 following incubation with BQ788, 22.2±4.1 pg/mg protein, vs. vehicle, 17.8±2.0, pg/mg protein. Because the marked effects of the ET-1-mediated ETA-R activation were observed following acute ETB-R blockade, it is not likely that they are due to increased ETA-R expression which has been reported in mesenteric arteries ETB-R deficient rats (45). Our studies further show a similar interaction between the activation of ETB-R and ETA-R in rats fed low sodium diet. The enhanced responsiveness of the renal mechanosensory nerves in the presence of the ETA-R antagonist was due to activation of ETB-R 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 diets are currently not known. ET-1 has high affinity to both ETA-R and ETB-R (8,51). 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-R and analgesic effect by activating ETB-R (25) have 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-R and ETA-R antagonists in rats fed high, normal and low sodium diet 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 diet. 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 renal medulla (13). The reasons for this apparent discrepancy are not clear. Although we can not exclude that the differential effects of ET-1 in vivo could be related to urinary ET-1 excretion being higher in high than low sodium diet rats, the similar nature of the responses to ETB-R and ETA-R antagonists, respectively, in vivo and in vitro 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 pelvises derived from rats fed high, normal and low sodium diet.

It is possible that the differential effects of ET on renal mechanosensory nerves could be explained by dietary sodium altering ET-R expression. Currently, there are few studies examining the effects of dietary sodium on ET-R 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 ETB-R but did not alter the ETA-R expression (59). In DOCA salt sensitive hypertension, there is an increased ETB-R 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 ETB-R 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 ETB-R in renal proximal tubular cells (66). These findings appear to contradict the notion of ETB-R 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 the activation of ET-1 on ETB-R and ETA-R.** The current studies suggest that activation of ETB-R and ETA-R on the responsiveness of renal sensory nerves involves an interaction between PGE₂ and ET-1. The ET-R 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 ETB-R (27) in non-neural renal tissue. ET-3, which has high affinity for ETB-R (51) increases urinary PGE₂ excretion in isolated perfused kidneys (56). Likewise, ET-3 induces COX-2 expression in astrocytes (39) which may be of relevance considering the location of the ETB-R on glia cells (48). On the other hand, studies on mechanical hypernociception indicate that activation of ETB-R may increase cAMP formation independent of PG-synthesis (6). ET-1 has also been shown to inhibit isoproterenol-induced activation of cAMP via activation of an ETA-R/pertussis toxin sensitive pathway in neural and non-neural cells (17,58). Conversely, ET-1 enhances capsaicin-induced CGRP release (12) possibly by activating ETA-R leading to increases in intracellular Ca++ and activation of protein kinase C (63) in cultured DRG. Whether these apparent conflicting findings are related to the ET-1 concentration and/or experimental conditions are currently not known.

In view of the stimulatory and inhibitory effects produced by the ETA-R and ETB-R antagonists in rats fed low and high sodium diet, respectively, we speculate that in conditions of high sodium diet, ET-1 mediated activation of ETB-R enhances the PGE₂-mediated activation of renal
sensory 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 PGE$_2$ synthesis in vivo. Conversely in conditions of low sodium diet, activation of ETA-R may suppress PGE$_2$-mediated activation of renal sensory nerves by preventing the PGE$_2$-mediated activation of cAMP, possibly involving mechanisms activated by ANG II (Fig. 8).

The restoration of the responsiveness of the renal sensory nerves to baseline in the presence of a combination of ETB-R and ETA-R 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 ET-R, 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(T$_9$-L$_1$), suggesting the presence of ET-1 in renal pelvic sensory nerves. The PGE$_2$-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 ETB-R antagonist in rats fed high sodium diet and enhanced by an ETA-R antagonist in rats fed 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 dietary intake, ET by activating ETB-R contributes to the enhanced responsiveness of renal mechanosensory nerves. Conversely, in conditions of low sodium dietary intake, ET by activating ETA-R 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.
ACKNOWLEDGMENT

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45. Perry MG, Molero MM, Giulumian AD, Katakam PVG, Pollock JS, Pollock DM and Fuchs LC. ETB receptor-deficient rats exhibit reduced contraction to ET-1 despite an increase in


FIGURE LEGENDS

Figure 1. Endothelin-1 (ET-1) levels in renal pelvic tissue, renal papillary tissue, renal cortical tissue and dorsal root ganglia (DRG)( T9-L1) in rats fed high (open bar) and low (hatched bar) sodium diet.

Figure 2. Effects of renal pelvic perfusion with vehicle (solid lines) and the ETB-R antagonist BQ788 (dashed lines) on the afferent renal nerve activity (ARNA) responses to increasing renal pelvic pressure 2.5 and 7.5 mmHg in rats fed high (A) and normal (B) sodium diet. * P<0.05, **P<0.01 vs. baseline; † P<0.05, ‡P<0.01 vs. ARNA responses to increasing renal pelvic pressure in the presence of vehicle.

Figure 3. Effects of renal pelvic perfusion with vehicle (solid lines) and the ETA-R antagonist BQ123 (dashed lines) on the ARNA responses to increasing renal pelvic pressure 2.5 and 7.5 mmHg in rats fed low (A) and normal (B) sodium diet. * P<0.05, **P<0.01 vs. baseline; † P<0.05, ‡P<0.01 vs. ARNA responses to increasing renal pelvic pressure in the presence of vehicle.

Figure 4. (A). Effects of renal pelvic perfusion with vehicle (open bar), BQ788 (hatched bar) and BQ788+BQ123 (cross hatched bar) on the ARNA responses to increased renal pelvic pressure 7.5 mmHg in rats fed high sodium diet. (B). Effects of renal pelvic perfusion with vehicle (open bar), BQ123 (hatched bar) and BQ788+BQ123 (cross hatched bar) on the ARNA responses to increased renal pelvic pressure 7.5 mmHg in rats fed low sodium diet. **P<0.01 vs. baseline; ‡ P<0.01 vs. the ARNA responses during pelvic perfusion with vehicle or BQ788+BQ123.

Figure 5. (A) High sodium diet rats: Effects of PGE₂, 0.03 μM, on substance P release from an
isolated renal pelvic wall preparation incubated in vehicle (solid line) or BQ788 (dashed line). (B) Low sodium diet rats: Effects of PGE<sub>2</sub>, 0.14 μM, on the substance P release from an isolated renal pelvic wall preparation incubated in vehicle or BQ788. **P<0.01 vs. control and recovery values. ‡ P<0.01 vs. PGE<sub>2</sub>-mediated increase in substance P release in the presence of BQ788. CNT, control; REC, recovery.

Figure 6. (A) Low sodium diet rats: Effects of PGE2, 0.14 μM, on substance P release from an isolated renal pelvic wall preparation incubated in vehicle (solid line) or BQ123 (dashed line). (B & C) Normal sodium diet rats: Effects of PGE<sub>2</sub> at 0.03 μM (B) and 0.14 μM (C) on substance P release from an isolated renal pelvic wall preparation incubated in vehicle or BQ123. **P<0.01 vs. control and recovery values. ‡ P<0.01 vs. PGE<sub>2</sub>-mediated increase in substance P release in the presence of vehicle.

Figure 7. (A) High sodium diet rats: Effects of PGE<sub>2</sub>, 0.03 μM, on substance P release from an isolated renal pelvic wall preparation incubated in BQ788 (dashed line) or BQ788+BQ123 (dotted line). (B) Low sodium diet rats: Effects of PGE<sub>2</sub>, 0.14 μM, on substance P release from an isolated renal pelvic wall preparation incubated in BQ123 (dashed line) and BQ123+BQ788 (dotted line). **P<0.01 vs. control and recovery values; ‡ P<0.01 vs. BQ788 and BQ123+BQ788 in rats fed high and low sodium diet, respectively.

Fig. 8. In the sequence of events elicited following an increase in renal pelvic pressure, PGE<sub>2</sub> exerts a stimulatory and ANG II an inhibitory effect on the activation of adenylyl cyclase. The interaction between PGE<sub>2</sub> and ANG II determines the level of activation of adenylyl 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 condition of high sodium dietary intake, ET-1 modulates the responsiveness of renal sensory nerves via activation of ETB-R by enhancing the PGE$_2$-mediated activation of adenylyl cyclase. Conversely in conditions of low dietary sodium intake, ET-1 via activation of ETA-R contributes to the ANG-II mediated suppression of the PGE$_2$-induced activation of adenylyl cyclase.
Table 1. ARNA responses to increasing renal pelvic renal pelvic pressure 7.5 mmHg three times in the presence of vehicle and BQ788 and BQ123 in rats fed high and low sodium diet, respectively.

<table>
<thead>
<tr>
<th>Renal pelvic perfusion</th>
<th>High Sodium Diet</th>
<th>Renal pelvic perfusion</th>
<th>Low Sodium Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARNA, %</td>
<td></td>
<td>ARNA%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>23±4**</td>
<td>Vehicle</td>
<td>7±2**</td>
</tr>
<tr>
<td>BQ788</td>
<td>3±3‡</td>
<td>BQ123</td>
<td>27±7‡</td>
</tr>
<tr>
<td>BQ788</td>
<td>9±2‡</td>
<td>BQ123</td>
<td>29±6‡</td>
</tr>
</tbody>
</table>

**P<0.01 vs baseline; ‡ P<0.01 vs. ARNA response during vehicle perfusion; both groups: n=6.
Table 2. ARNA responses to increasing renal pelvic pressure 7.5 mmHg three times in the presence of vehicle in rats fed high and low sodium diet (time controls).

<table>
<thead>
<tr>
<th>Renal Pelvic Perfusion</th>
<th>High Sodium Diet</th>
<th>Low Sodium diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARNA%</td>
<td>ARNA, %</td>
</tr>
<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>

* P<0.05, **P<0.01 vs. baseline; both groups: n=10.
Table 3. PGE$_2$ mediated release of substance P from isolated renal pelvises incubated in vehicle or BQ123. Pelvises were derived from rats fed high sodium diet.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>PGE$_2$, 0.03 μM</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Substance P release, pg/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.4±0.6</td>
<td>16.8±2.2**</td>
<td>5.6±1.0</td>
</tr>
<tr>
<td>BQ123</td>
<td>8.5±2.2</td>
<td>17.4±3.4**</td>
<td>7.9±2.4</td>
</tr>
</tbody>
</table>

**P<0.01 vs. average of control and recovery, N=6
Figure 1

Endothelin -1

Pelvis

Papilla

Cortex

DRG

ET-1 pg/mg protein

High NaCl diet (n=11)

Low NaCl diet (n=10)
Figure 2

Effects of ETB-R Antagonist BQ788 on the ARNA Responses to Increased Renal Pelvic Pressure

- **A** High NaCl diet
  - Vehicle
  - BQ788

- **B** Normal NaCl diet
  - Vehicle
  - BQ788

ARNA response, %

Renal Pelvic Pressure, mmHg

n=10

n=6
Figure 3

Effects of ETA-R Antagonist BQ123 on the ARNA Responses to Increased Renal Pelvic Pressure

A. Low NaCl diet

B. Normal NaCl diet

Vehicle

BQ123

Significance:

* p < 0.05

** p < 0.01

† p < 0.001
Figure 4

Effects of ETB-R (left) and ETA-R (right) Antagonists and Their Combination on the ARNA Responses to Increased Renal Pelvic Pressure

A  
High NaCl diet

B  
Low NaCl diet

<table>
<thead>
<tr>
<th></th>
<th>ARNA response, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>**</td>
</tr>
<tr>
<td>BQ788</td>
<td></td>
</tr>
<tr>
<td>BQ788+ BQ123</td>
<td>**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>ARNA response, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
</tr>
<tr>
<td>BQ123</td>
<td>*</td>
</tr>
<tr>
<td>BQ788+ BQ123</td>
<td>**</td>
</tr>
</tbody>
</table>

n=8, n=13
Figure 5

Effects of an ETB-R Antagonist on PGE₂-mediated Substance P Release in Rats on High and Normal Sodium Diets

A

High NaCl diet
PGE₂, 0.03 μM

B

Normal NaCl diet
PGE₂, 0.14 μM

SP release, pg/min

CNT  PGE₂  REC

n=11

n=8

— Vehicle
— BQ788

**
Figure 6
Effects of ETA-R Antagonist on PGE$_2$-mediated Substance P Release in Rats on Low and Normal Sodium Diets

A  
Low NaCl diet  
PGE$_2$ 0.14 μM

B  
Normal NaCl diet  
PGE$_2$ 0.14 μM

C  
Normal NaCl diet  
PGE$_2$ 0.03 μM

SP release, pg/min

- • Vehicle  
- ○ BQ123

n=15  
n=8  
n=8
Figure 7
Effects of ETB-R and ETA-R Antagonists and their combination on PGE$_2$-mediated Substance P Release in rats on high and low sodium diets

A
High NaCl diet
PGE$_2$, 0.03 μM

- - BQ788
- - - - BQ788+BQ123

B
Low NaCl diet
PGE$_2$, 0.14 μM

- - BQ123
- - - - BQ123+BQ788

SP release, pg/min

CNT  PGE$_2$  REC

n=14  n=13
Figure 8

HYPOTHESIS

\[ \uparrow \text{Renal Pelvic Pressure} \]
\[ \text{(B}_{2}\text{ receptors)} \]

\[ \downarrow \text{COX-2} \quad \text{PKC} \]

\[ \downarrow \text{PGE}_{2} \]

\[ \text{ETB-R} \quad \text{?} \quad \text{+} \quad \text{Adenylyl Cyclase} \]

\[ \text{Angiotensin II} \quad \text{?} \quad \text{ETA-R} \]

\[ \rightarrow \text{cAMP} \]

\[ \text{Substance P} \quad \text{(Ca}^{++}\text{-dependent)} \rightarrow \text{PKA} \]

\[ \uparrow \text{Afferent Renal Nerve Activity} \]