Endothelin Contributes to Blunted Renal Autoregulation Observed with a High Salt Diet

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Abstract

Autoregulation of renal blood flow (RBF) is an essential function of the renal microcirculation that has been previously shown to be blunted by excessive dietary salt. Endogenous endothelin 1 (ET-1) is increased following a high salt (HS) diet and contributes to the control of RBF but the differential effects of ET-1 on renal microvessel autoregulation in response to HS remain to be established. We hypothesized that a HS diet increases endothelin receptor activation in normal Sprague-Dawley rats and blunts autoregulation of RBF. The role of ET-1 in the blunted autoregulation produced by a HS diet was assessed in vitro and in vivo using the blood-perfused juxtamedullary nephron preparation and anesthetized rats, respectively. Using highly selective antagonists, we observed that blockade of either ET_A or ET_B receptors was sufficient to restore normal autoregulatory behavior in afferent arterioles from HS fed rats. Additionally, normal autoregulatory behavior was restored in vivo in HS fed rats by simultaneous ET_A and ET_B receptor blockade whereas blockade of ET_B receptors alone showed significant improvement of normal autoregulation of RBF. Consistent with this observation, autoregulation of RBF in ET_B receptor deficient rats fed HS was similar to both ET_B-deficient rats and transgenic control rats on NS diets. These data support the hypothesis that endogenous ET-1, working through ET_B and possibly ET_A receptors, contributes to the blunted renal autoregulatory behavior in rats fed a HS diet.
Keywords

ET\(_A\) receptors, ET\(_B\) receptors, afferent arterioles, BQ-123, BQ-788
Introduction

Excess salt consumption in western diets is prevalent, with Americans consuming an average of twice the American Heart Association recommended amount of sodium per day (2). Reducing dietary sodium is linked to significant reductions in systolic blood pressure in hypertensive humans (2; 24; 51). Excess dietary NaCl can also increase systolic blood pressure in salt-sensitive humans (2; 5) and animals and can contribute to hypertensive renal injury (1). An important defense mechanism for prevention of hypertensive kidney injury is the inherent ability to adjust preglomerular renal vascular resistance to autoregulate renal blood flow (RBF) and glomerular capillary pressure in the face of changes in renal arterial pressure (4; 28; 31; 40; 45). Autoregulation involves two or more mechanisms working in concert to stabilize RBF and glomerular filtration rate when challenged with fluctuations in renal arterial pressure (31; 40; 45). For example, increases in renal arterial pressure result in renal vasoconstriction, largely involving afferent arterioles. This increase in renal vascular resistance offsets the increase in arterial pressure to maintain a stable RBF and glomerular filtration rate. The two best understood mechanisms of renal autoregulation are the myogenic response of preglomerular vascular smooth muscle and the tubuloglomerular feedback mechanism which influences afferent arteriole resistance via signaling from the macula densa cells of the distal nephron (13; 31; 43; 45; 57). While the existence of the autoregulatory phenomenon is well accepted, the effect of salt consumption on autoregulatory behavior is not well understood.
Since its discovery in the 1980’s (25; 62), endothelin has been shown to strongly influence renal regulation of salt and water homeostasis (35; 44; 53). Additionally, HS intake reportedly increases plasma endothelin 1 (ET-1) modestly in rats and humans (12; 39). Endothelin is produced by many renal cell types and is a potent vasoconstrictor with both intra-renal and extra-renal effects (20; 29; 35-37; 41; 59). Studies show that HS intake increases ET-1 levels in the vascular wall (60) and stimulates ET-1 release in the renal medulla (6).

Endothelin receptors are divided into two main subtypes named $\text{ET}_A$ and $\text{ET}_B$ (35; 36; 42). Both receptor subtypes are G-protein coupled and they are differentially expressed by renal tissue (35; 36; 42). ET-1 induces a long lasting vasoconstriction of afferent and efferent arterioles through activation of $\text{ET}_A$ receptors located on vascular smooth muscle cells (14; 22; 27; 29; 38). $\text{ET}_B$ receptor activation can exert vasoconstrictor and/or vasodilatory influences depending on expression of $\text{ET}_B$ receptors by endothelium or vascular smooth muscle. $\text{ET}_B$ receptors are expressed by the renal microvasculature and expression increases in kidneys from rats fed a HS diet (14; 29; 56). Nevertheless, the relative expression levels between the endothelium and vascular smooth muscle tissues are not clear. Conversely, another study showed that a HS diet increases the ratio of $\text{ET}_A$ to $\text{ET}_B$ receptors in the kidneys of spontaneously hypertensive rats (53). Endothelin has been shown to play a role in autoregulation through an $\text{ET}_B$ dependent mechanism that is purported to signal through NO and modulates the myogenic response (58). Taken together these observations led to the hypothesis that a HS diet increases endothelin receptor activation in normal Sprague-
Dawley rats and blunts autoregulation of RBF. Experiments were performed to
determine if increased salt intake leads to an endothelin receptor-dependent decrease
in afferent arteriole autoregulatory behavior in vitro and if this effect could be
demonstrated in vivo relating to whole kidney autoregulation of RBF.
Methods

Animals: Experiments were performed using male Sprague-Dawley rats (270–330g; Charles River Laboratories, Raleigh, NC). All animals were cared for in accordance with National Institutes of Health guidelines, and all procedures were approved by the Georgia Regents University and UAB Institutional Animal Care and Use Committees.

Diet: Rats were fed either a normal salt diet (NS; 0.8% NaCl) or high salt (HS; 8% NaCl) diet for 14-days prior to each experiment and had ad libitum access to tap water.

In vitro blood-perfused juxtamedullary nephron preparation: Videomicroscopy experiments were performed in vitro using the blood-perfused juxtamedullary nephron technique, as previously described (19). The ETA receptor antagonist, BQ-123 (1.0 μM; Sigma Aldrich, St. Louis, Mo.), or the ETB receptor antagonist, BQ-788 (1.0 μM; Sigma) were added to the reconstituted blood used to perfuse the prepared kidney. Experiments were designed to assess autoregulatory behavior, defined as the change in afferent arteriole diameter induced by step changes in renal perfusion pressure (pressure/diameter relationship). To assess autoregulatory function, afferent arteriolar diameters were measured during a control period at renal perfusion pressure of 100 mmHg and then at pressures ranging from 65 to 170 mmHg stepped in 15-mmHg increments over successive 5-minute periods. Afferent arteriolar diameter was calculated from the average of measurements taken at a single site at 12-second intervals during the last two minutes of the five minute pressure step.
In vivo autoregulation of RBF: Rats were anesthetized with sodium pentobarbital (50 mg/kg; i.p.; Hospira Inc., Lake Forest, IL, USA) and placed on a servo-controlled heating table to maintain body temperature (37–38°C) as measured by a rectal probe (Physitemp, Clifton, NJ). A tracheotomy tube (PE-205) was inserted into the trachea to ensure a patent airway. The left femoral artery and right carotid artery were cannulated (PE-50) for continuous recording of arterial pressure both below (femoral) and above (carotid) an adjustable aortic screw clamp. Two catheters were inserted into the right jugular vein for infusion of maintenance solutions and for infusion endothelin agonists and antagonists. The maintenance catheter (PE-50 tubing) delivers 6% bovine serum albumin (Sigma) in phosphate buffered saline infused at 20 μl/min during surgery and then reduced to 10 μl/min during the experimental periods. The second jugular catheter (PE-10) was used for infusion of BQ-123 (30 nmoles/kg/min; 10 μl/min; PBS) or BQ-788 (30 nmoles/kg/min; 10 μl/min; 7% EtOH) for blockade of ET_A and ET_B receptors, respectively. In all cases, the total infusion volume during the experimental periods was 20 μl/min.

The abdomen was opened by a midline incision. A segment of the aorta between the left and right renal arteries was separated from the surrounding connective tissue and vena cava. An adjustable screw clamp vascular occluder (Vestavia Scientific, Vestavia Hills, AL) was placed around the aorta to adjust left renal arterial pressure (RAP) as monitored by the femoral artery cannula. The left renal artery was separated from the renal vein and an ultrasonic flow probe (MA1PRB, Transonic Systems, Ithaca, NY) was installed to measure whole kidney RBF. Arterial pressure (carotid and femoral), heart
rate, and RBF were continuously recorded by an eight-channel PowerLab (ADInstruments, Colorado Springs, CO). Upon completion of the surgical procedures the animals were allowed to stabilize for 60 min before beginning experiments.

Assessment of autoregulation:

Whole kidney autoregulation experiments were performed to assess the impact of step reductions in RAP on RBF. The protocol used to assess whole kidney autoregulation of RBF consisted of a series of reductions in RAP to determine the RBF response of animals on normal and HS before and after endothelin receptor blockade. Control or baseline autoregulatory function was determined prior to administration of endothelin receptor antagonists with a 10-minute control period to establish baseline mean arterial pressure (MAP) and RBF; followed by successive reductions of RAP from ambient to 105, 95 and 85 mmHg. The vascular occluder was tightened until femoral artery pressure (representing renal arterial pressure) declined to each target pressure where it was maintained for 5 minutes. Following the period at 85 mmHg, the clamp was released to allow RAP to return to ambient. After a 10 minute recovery period, selective $E_{TA}$ and/or $E_{TB}$ receptor antagonist or vehicle (7% EtOH in PBS) infusion was started and an equilibration period was allowed for blood pressure to return to at least 108 mmHg. Once blood pressure and RBF stabilized in the presence of the endothelin receptor antagonist (or vehicle), RAP was again reduced from ambient to 105, 95 and 85 mmHg followed by a 10 minute recovery period. After this recovery period, we administered a 10 pmole bolus of ET-1 to verify endothelin receptor blockade. The
response to bolus injection of ET-1 was calculated as the peak carotid artery pressure minus the pressure just prior to ET-1 injection.

A separate series of in vivo autoregulation studies was performed using ET$_B$-receptor deficient rats, which are on the spotting lethal rat background (17). The spotting-lethal rat carries a natural 301-bp deletion in the ET$_B$ receptor gene, resulting in expression of dysfunctional ET$_B$ receptors. Rats that are homozygous (sl/sl) for this mutation exhibit a lethal phenotype of congenital intestinal agangliosis. In 1998, Gariepy et al used a dopamine-β-hydroxylase promoter to direct transgenic ET$_B$ receptor expression only in the enteric nervous system, thereby rescuing the rats from the intestinal defect. We performed autoregulation studies using these ET$_B$-receptor deficient rats, to complement those above in assessing the role of ET$_B$ receptors on the autoregulatory impairment observed in rats on a HS diet. Littermates with functional copies of the ET$_B$ gene and positive for the transgene were used as controls (Tg control).

Autoregulatory index (AI) was calculated according to following formula: AI for RBF = $\frac{(RBF_1 - RBF_2)/RBF_1}{(RAP_1 - RAP_2)/RAP_1}$, as previously reported (15; 19; 49; 50). RBF$_1$ and RAP$_1$ indicate the values during the previous period while RBF$_2$ and RAP$_2$ describe the values during that period of pressure reduction. An AI of zero means perfect autoregulation and an AI approaching one depicts impaired autoregulatory capability.
**Statistical analyses:** Data are presented as the mean ± SEM for n = 7–12 rats per group. *In vivo* RBF data and *in vitro* juxtamedullary nephron data were analyzed by 2-way ANOVA for repeated measures followed by (Bonferroni’s) post hoc test to compare between groups. AI data were also analyzed by 2-way ANOVA for repeated measures followed by (Bonferroni’s) post hoc test to compare between groups. The response to bolus injection of ET-1 was analyzed using a paired t-test. $P \leq 0.05$ was considered statistically significant for all analyses.
Results

**ET<sub>A</sub> receptor blockade restores autoregulatory behavior of juxtamedullary afferent arterioles from HS rats**

Acute ET<sub>A</sub> receptor blockade with BQ-123 had no significant effect on baseline afferent arteriole diameter in kidneys from NS (15.8 ± 0.1 μm vs. 14.2 ± 0.4 μm with BQ-123) or HS rats (14.5 ± 0.9 μm vs. 14.1 ± 0.5 μm with BQ-123) at a perfusion pressure of 100 mmHg (Fig. 1). Figure 2 illustrates the effect of acute ET<sub>A</sub> receptor blockade on afferent arteriolar autoregulatory behavior. Increasing perfusion pressure from 65 to 170 mmHg resulted in a significant reduction in afferent arteriolar diameter to 74 ± 2% of baseline in kidneys from NS rats while the diameters of arterioles from HS rats (15.3 ± 1.4 μm) did not decrease significantly over the same pressure range (Figs. 2A and 2B). Acute ET<sub>A</sub> blockade with BQ-123 normalized afferent arteriolar autoregulatory behavior in kidneys from HS rats such that arteriolar diameter declined to 12.6 ± 0.7 μm or 82 ± 3% of control when perfusion pressure was increased to 170 mmHg (Figs. 2A and 2B). ET<sub>A</sub> blockade did not alter the autoregulatory response observed in NS kidneys (Figs. 2A and 2B).

**ET<sub>B</sub> receptor blockade restores autoregulatory behavior of afferent arterioles from HS rats**

Baseline afferent arteriole diameters were slightly smaller in NS rat kidneys during acute ET<sub>B</sub> receptor blockade with BQ-788 (15.8 ± 0.1 μm vs. 13.2 ± 0.4 μm BQ-788, P<0.05; Fig. 1) but BQ-788 had no significant effect on baseline diameter in HS kidneys (14.5 ± 0.9 μm vs. 13.9 ± 0.5 μm with BQ-788). Figure 3 illustrates the effect of acute ET<sub>B</sub>
receptor blockade on afferent arteriolar autoregulatory behavior. BQ-788 treatment
normalized afferent arteriolar autoregulatory behavior in kidneys from HS rats such that
arteriolar diameter declined to 12.3 ± 0.7 μm or 84 ± 2% of control when perfusion
pressure was increased to 170 mmHg (Figs. 3A and 3B). Though ET_B receptor
blockade slightly reduced baseline arteriolar diameter in kidneys from NS rats, the
autoregulatory response was similar to that observed in control kidneys (Figs. 3A and
3B). This might reflect a tonic vasodilatory influence of ET_B receptors on baseline
afferent arteriole diameter.

**HS blunts autoregulation of whole kidney RBF**

All groups of rats used for in vivo studies exhibited statistically similar body weights on
the day of the experiment. Body weights averaged between 298 ± 5 to 305 ± 4 grams
indicating similar levels of food consumption and nutrition over the 14 day feeding
schedule. Figure 4 illustrates the effect of reducing RAP on RBF. RBF was significantly
greater in HS rats (Fig. 4A; 12.5 ± 0.6 ml/min vs. 9.8 ± 0.6 ml/min for NS rats (P < 0.05))
under baseline conditions where the kidneys were perfused at ambient RAP. When the
aortic screw clamp was tightened to reduce RAP to 105 mmHg, RBF declined by 5 ±
1% (Fig. 4B) in HS rats from a baseline of 12.6 ± 0.6 to 11.8 ± 0.6 ml/min, while it
increased slightly but significantly by 2 ± 1% (from 9.8 ± 0.6 to 10.0 ± 0.6 ml/min; P <
0.05; Fig. 4A) in kidneys from NS rats. This trend continued when RAP was further
reduced from 105 to 95 mmHg. RBF declined 16 ± 3% in HS from a baseline of 12.6 ±
0.6 to 10.4 ± 0.6 ml/min compared to NS rats which exhibited a 4 ± 2% decrease (from
9.8 ± 0.6 to 9.4 ± 0.6 ml/min; P < 0.05; Fig. 4A). The AI for NS rats averaged -0.54 ±
0.35 and 0.22 ± 0.11 for the reduction in pressure from ambient to 105 mmHg and from ambient to 95 mmHg; respectively, indicating good autoregulatory capacity for what is viewed as the normal autoregulatory range. However, the AI for HS rats was 0.50 ± 0.15 and 0.80 ± 0.14 over the same pressure spans, indicating significantly compromised autoregulatory function (Fig. 5; P < 0.05).

**Dual ETₐ/ETₜ receptor blockade restores blunted autoregulation of RBF in HS rats**

Figure 4 also illustrates the effects of acute blockade of both ETₐ and ETₜ on RBF autoregulation. Prior to endothelin receptor blockade, RBF on NS rats was efficiently autoregulated over the RAP range of ambient to 95 mmHg whereas that of HS rats was not (Fig. 4). Both HS and NS rats receiving combined treatment with BQ-123 and BQ-788 exhibited similar baseline RBF’s despite the fact that MAP decreased significantly in both groups following endothelin receptor blocker administration (Fig. 4A; P < 0.05). When the screw clamp was tightened to reduce RAP to 105 mmHg in HS+BQ-123/BQ-788 rats, RBF actually increased slightly by 3 ± 2% from a baseline of 11.7 ± 0.6 to 11.9 ± 0.6 ml/min compared to before ETₐ/ETₜ blockade when RBF decreased by 5 ± 1% (from 12.6 ± 0.6 to 11.8 ± 0.6 ml/min; P < 0.05; Figs. 4A and 4B). Further reduction of RAP to 95 mmHg led to a slight decline in RBF to 96 ± 3% of baseline in HS+BQ-123/BQ-788 rats from 11.9 ± 0.6 to 11.4 ± 0.6 ml/min compared to before ETₐ/ETₜ blockade where RBF decreased to 84% of baseline (from 11.7 ± 0.6 to 10.4 ± 0.6 ml/min; P < 0.05; Figs. 4A and 4B). In comparing the different groups after normalizing for baseline RBF (Fig 4B), there is a clear separation of RBF autoregulation between NS and HS rats. Furthermore, dual ETₐ/ETₜ receptor blockade returns the
autoregulatory pressure/flow relationship to normal. The AI for the pressure change from ambient to 105 mmHg and ambient to 95 mmHg for HS+BQ-123/BQ-788 rats averaged -0.50 ± 0.15 and 0.80 ± 0.14; respectively before endothelin receptor blockade and -0.33 ± 0.14 and 0.23 ± 0.15 after combined endothelin receptor blockade (Fig. 5). The AI observed in the HS+BQ-123/BQ-788 during endothelin receptor blockade was similar to that observed in NS rats before and during combined endothelin receptor blockade (NS+BQ-123/BQ-788).

Control experiments showed no vehicle effect on autoregulatory behavior. The AI for the pressure change from ambient to 105 mmHg and ambient to 95 mmHg for HS vehicle controls averaged 0.46 ± 0.16 and 0.69 ± 0.04 before vehicle infusion and 0.76 ± 0.19 and 0.79 ± 0.07 during vehicle infusion (Fig. 5).

Effect of ET\textsubscript{A} receptor blockade on blunted RBF autoregulation in HS rats

Figures 6A and 6B illustrate the effects of selective ET\textsubscript{A} receptor blockade with BQ-123 on RBF autoregulation. ET\textsubscript{A} blockade reduced MAP from 119 ± 1 to 111 ± 1 mmHg (Fig. 6A; \(P < 0.05\)) but had little effect on RBF autoregulation. When the aortic clamp was tightened to reduce RAP to approximately 105 mmHg in HS+BQ-123 rats, RBF decreased by approximately 2% from a baseline of 11.9 ± 0.2 to 11.7 ± 0.6 ml/min compared to an 8 ± 3% decrease (from 12.6 ± 0.6 to 11.8 ± 0.6 ml/min; \(P < 0.05\); Fig. 6) before ET\textsubscript{A} receptor blockade. Further reducing RAP to 95 mmHg caused RBF to fall by 12 ± 8% of baseline (11.7 ± 0.6 to 10.4 ± 0.8 ml/min) in HS+BQ-123 rats compared to a 16 ± 3% decrease (from 11.8 ± 0.6 to 10.4 ± 0.6 ml/min; \(P < 0.05\); Fig. 6) before
ET\textsubscript{A} blockade. The AI for the pressure change from ambient to 105 mmHg and ambient to 95 mmHg in these HS rats averaged 0.92 ± 0.39 and 0.93 ± 0.12; respectively before BQ-123 and 0.21 ± 0.25 and 1.01 ± 0.41 during BQ-123 treatment over the same pressure span (Fig. 5) suggesting some improvement in autoregulatory control at the higher pressures but no significant improvement in autoregulatory performance over the broader pressure span.

**Effect of acute ET\textsubscript{B} receptor blockade on blunted RBF autoregulation in HS rats**

Figures 6C and 6D illustrate the effects of acute blockade of ET\textsubscript{B} receptors with BQ-788 on RBF autoregulation. BQ-788 had little effect on MAP but reduced baseline RBF from 12.9 ± 0.2 to 9.2 ± 0.2 ml/min (Fig. 6C; P < 0.05). Reducing RAP from ambient to 105 mmHg resulted in a decline in RBF of approximately 3 ± 2% from a baseline of 9.2 ± 0.2 to 8.9 ± 0.3 ml/min in HS rats with BQ-788 compared to 9 ± 3% decrease (from 12.7 ± 0.2 to 11.6 ± 0.2 ml/min; P < 0.05; Fig. 6A) before ET\textsubscript{A} receptor blockade. Similarly, when RAP was reduced to 95 mmHg, RBF only declined by 8 ± 3% in HS+BQ-788 compared to a 16 ± 3% decrease observed before ET\textsubscript{A} receptor blockade (P < 0.05; Fig. 6A). The AI for the pressure change from ambient to 105 mmHg and ambient to 95 mmHg in HS rats averaged 0.78 ± 0.21 and 0.83 ± 0.15 before ET\textsubscript{B} receptor blocker treatment and 0.18 ± 0.32 and 0.38 ± 0.19 during BQ-788 administration (Fig. 5; P < 0.05).

**Effect of an ET-1 bolus injection on carotid arterial pressure**
At the end of each receptor blocker protocol, rats received a bolus injection of ET-1 (10 pmoles) to determine the effect on MAP measured with the carotid artery cannula and the results are shown in Figure 7. Bolus injection of ET-1 in NS+BQ123/BQ788 revealed no significant change (P = 0.25) in MAP indicating good blockade of ET<sub>A</sub> and ET<sub>B</sub> receptors. Vehicle control rats fed a HS diet responded to the ET-1 bolus with a significant increase in MAP of approximately 10 mmHg (P < 0.05). Similarly, MAP also increased approximately 13 mmHg in HS rats receiving BQ-788 alone (P < 0.05) suggesting that the bulk of the ET-1 effect is through ET<sub>A</sub> receptor activation. No significant change in MAP was noted in either the HS+BQ123/BQ788 (P = 0.29) or the HS+BQ-123 (P = 0.90) treated rats in response to the ET-1 bolus.

**ET<sub>B</sub> receptor deficiency preserves autoregulation of RBF in HS rats**

MAP under anesthesia was similar in Tg control groups and averaged 114 ± 2 and 115 ± 4 mmHg in rats fed NS and HS, respectively. MAP was significantly higher in the ET<sub>B</sub>-deficient rats than their controls and averaged 132 ± 5 and 131 ± 5 mmHg in the NS and HS rats, respectively. Baseline RBF was greater in the Tg control rats eating HS and averaged 9.2 ± 0.6 and 13.6 ± 0.9 ml/min in the NS and HS groups, respectively. Baseline RBF of ET<sub>B</sub>-deficient rats averaged 7.4 ± 0.6 and 7.0 ± 0.5 ml/min in the NS and HS groups, respectively. Notably, HS did not increase baseline RBF in ET<sub>B</sub>-deficient rats as it did in Tg control rats. Figure 8 illustrates the effects of a HS diet on autoregulation of RBF in Tg controls and ET<sub>B</sub> deficient rats. The autoregulatory response observed from ET<sub>B</sub>-deficient rats on NS and HS diets and the response observed in Tg control rats on NS were normal, yielding AI’s of 0.26 ± 0.14, 0.12 ± 0.2
and 0.18 ± 0.1, respectively. In contrast, HS diet significantly impaired autoregulatory behavior in the Tg control group on HS yielding an AI of 0.63 ± 0.08 (P<0.05 vs. NS).
Discussion

This study employed three approaches to assess the impact of high dietary salt and endothelin receptors on autoregulation including the in vitro blood perfused juxtamedullary nephron technique and whole kidney autoregulation using anesthetized rats. The whole kidney studies included use of normal rats and rats with a naturally occurring ET$_B$ receptor deficiency (17). The results of the studies demonstrate that renal autoregulatory behavior is blunted in rats fed a HS diet for 14 days, consistent with our previous report (15). This blunted autoregulatory capability involves endothelin receptor signaling primarily through ET$_B$ receptors but may also involve ET$_A$ receptors. This is supported by the observations that acute pharmacological blockade of either ET$_A$ or ET$_B$ receptors with BQ-123 or BQ-788, respectively completely normalizes autoregulatory behavior of juxtamedullary afferent arterioles in vitro. Furthermore, rats maintained on HS and given an acute infusion of the ET$_B$ antagonist; BQ-788, significantly improved whole kidney autoregulation of RBF. Notably, in vivo treatment with the ET$_A$ antagonist, BQ-123, evoked no detectable improvement in the blunted autoregulation observed in HS rats when assessed over the larger pressure range from ambient MAP to 95 mmHg but did show improvement over the range of ambient to 105 mmHg such that the AI was similar to NS controls and different from HS rats. Additionally, data from ET$_B$ deficient rats support the hypothesis that excessive dietary salt consumption can lead to blunted renal autoregulation derived in part through endothelin activity.
The in vitro blood perfused juxtamedullary nephron technique has been used to study the control of glomerular and renal microvascular function since 1984 (8). Some of the earliest studies with this approach demonstrated that juxtamedullary glomerular capillary pressure and intravascular pressures along the juxtamedullary preglomerular vascular tree were autoregulated in response to step changes in renal perfusion pressure (7; 26). Later work demonstrated that blood flow through a single juxtamedullary afferent arteriole was also autoregulated in response to a step change in perfusion pressure and that both TGF and myogenic inputs were involved in the pressure-induced resistance adjustments (61). The work from these published reports establish that the in vitro blood perfused juxtamedullary nephron technique is suitable for measuring juxtamedullary afferent arteriolar autoregulatory behavior.

In the current study we used a broad pressure range extending from 65 to 170 mmHg and observed pressure-mediated vasoconstriction in NS rats over the entire range. This is consistent with good autoregulation in this nephron population. Some might consider that the pressure sensitivity observed at the lower pressure range is greater than would be predicted from whole kidney autoregulation in vivo, but it should be remembered that this is an in vitro technique that has demonstrated autoregulation of pressure and flow in response to pressure stimuli (7; 26; 61). In addition, whole kidney autoregulatory measurements of renal blood flow reflect renal vascular responses from the entire preglomerular vascular tree throughout the renal cortex rather than the response of a single arteriole in a single region of the kidney. Indeed Källog et al reported that inner cortical autoregulatory behavior was more pronounced than outer
cortical autoregulation (33). Consequently, as one spans the autoregulatory range, autoregulatory capacity would be exhausted in some nephrons sooner than in others yielding an averaged RBF autoregulation profile where the RBF plateau would begin to roll into a more passive whole kidney pressure/flow relationship earlier in the pressure range. Comparing this to published whole kidney autoregulation data reveals that the estimated lower limit of autoregulatory capacity (arbitrarily set at 90% of baseline RBF) varies from approximately 75 to 97 mmHg in anesthetized rat studies (3; 9-11; 19; 30; 49; 50; 52). Thus the in vitro data are in good qualitative agreement with in vivo data within the typical autoregulatory range.

The ability of high dietary salt to attenuate myogenic reactivity in large skeletal muscle arterioles of normotensive rats has been known for some time (46; 47). There is convincing evidence in animal models that excessive salt consumption can lead to renal microvascular dysfunction, and glomerular injury (1; 15). We recently demonstrated that the blunted renal autoregulatory response observed with afferent arterioles at the whole kidney level in response to high dietary salt is reversed by acute treatment with the NADPH-oxidase inhibitor and/or scavenger of reactive oxygen species, apocynin (15). HS diets are believed to reduce NO bioavailability and promote enhanced accumulation of reactive oxygen species (48). Future studies will have to explore whether our findings that ET_{B} receptors reduce autoregulatory reactivity under HS conditions are related to oxidative stress.
In vitro data presented here show that acute blockade of either ET_A or ET_B receptors normalized afferent arteriolar diameter responses to changes in renal perfusion pressure. Given the highly selective nature of these antagonists, it is interesting that either receptor antagonist was effective in this setting. One consideration for this unexpected observation is that the renal endothelin receptor system is more complex than the classical views of simple receptor/ligand activation. The idea that ET_A and ET_B receptors may interact with one another to yield complex physiological outcomes has been suggested before. Competitive binding studies suggested that ET_B receptor selective ligands competed with ET-1 for binding only when a selective ET_A agonist was present (21). There is evidence in astrocytes that both ET_A and ET_B receptors had to be blocked in order to prevent ET-1 clearance (23). With respect to the renal circulation, Just et al concluded that stimulation of ET_B receptors alone led to renal vasoconstriction but during co-stimulation of both receptor subtypes, ET_B agonists produced a renal vasodilation (32). They concluded that there must be a more complex interaction between ET_A and ET_B receptors that was more than additive. Furthermore, in the juxtamedullary nephron preparation, we reported a somewhat puzzling finding that both ET_A and ET_B receptor antagonists equally inhibited afferent arteriolar vasoconstriction produced by low doses of ET-1 (29). Receptor interaction is possible as ET_A and ET_B receptors are postulated to form homodimers and heterodimers in transfected cells (18). This theory is interesting and clearly requires further study but it could explain the essentially equivalent beneficial effects of ET_A or ET_B receptor blockers on autoregulatory responses of blood perfused juxtamedullary nephron afferent arterioles in vitro. Receptor cooperativity and post-receptor/G-protein signaling
could also account for these findings but more work is required to explain this phenomenon.

We extended the in vitro findings by assessing the effects of endothelin receptor antagonists on whole kidney autoregulation of RBF. Consistent with the in vitro data and with our previous whole kidney data (15), we found that whole kidney autoregulation was significantly impaired in kidneys from rats fed HS. The whole kidney results with endothelin receptor blockers are qualitatively consistent with in vitro data in that acute ET$_B$ receptor blockade significantly improved autoregulation of RBF and autoregulation was preserved in ET$_B$-deficient rats fed HS. Joint blockade of both ET$_A$ and ET$_B$ receptors effectively restored normal autoregulation in HS conditions in vivo. Interestingly, results with ET$_A$ receptor blockade were mixed. ET$_A$ receptor blockade conferred some restoration of autoregulatory control at higher pressures (ambient to 105 mmHg) but over the broader pressure range (ambient to 95 mmHg) there was no detectable effect on whole kidney autoregulation of RBF in vivo. The explanation for this is difference in effectiveness is unclear but could reflect a number of important differences between the two experimental settings. Clearly one set of data are collected in vivo and the other in vitro. Whole kidney hemodynamics data reflect the microvascular outcomes of all of the vascular elements within the kidney whereas the in vitro findings focus on the juxtamedullary afferent arterioles. If receptor interactions do influence the renal hemodynamic response to endothelin, there could be regional differences in how those interactions are manifested.
Another possible explanation is that the set points for the lower limit of autoregulation may have changed under HS conditions in vivo. This explanation seems unlikely though since the AI patterns were similar across groups over the ambient to 105 mmHg and the ambient to 95 mmHg ranges. In both cases autoregulation of RBF was impaired in the HS group and acute joint ET\textsubscript{A}/ET\textsubscript{B} receptor blockade normalized it. In addition, AI was normal during ET\textsubscript{B} receptor blockade and in ET\textsubscript{B} deficient rats. Interestingly, ET\textsubscript{A} receptor blockade exhibited mixed effectiveness with good restoration of RBF autoregulation at higher pressures (ambient to 105 mmHg) and poorer effectiveness at lower pressures (ambient – 95 mmHg). Accordingly, overall resetting of the lower limit of autoregulation does not explain these findings. The data also seem to reveal a modulatory role for ET\textsubscript{A} receptors or an unexplained ET\textsubscript{A}/ET\textsubscript{B} receptor interaction that collectively influences autoregulatory function in HS conditions.

In comparing the in vitro findings with the in vivo findings, another similarity emerges. Based on the in vitro data presented, the pressure/diameter response of the HS group already begins deviating from the control response at perfusion pressures of about 95 mmHg or even 80 mmHg and is more pronounced at 110 mmHg. This leads to a clearly shallower pressure/diameter relationship in the HS animals compared to the NS controls at perfusion pressures that approximate those lower renal arterial pressures used in the in vivo experiments. The relatively unchanging arteriolar diameter from the range of 140 to 95 mmHg would result in a more passive pressure/flow relationship in vivo and would manifest as a decline in RBF as RAP is reduced over that range. Indeed, the existence of functional impairment in the in vivo autoregulatory response is
supported by the fact that the AI reflecting the autoregulatory range from ambient to 95 mmHg RAP is significantly greater in the HS group than the NS group. Accordingly, there is good qualitative agreement between the trends observed in the in vitro setting and those manifested in vivo. Because of the limitations inherent in the in vivo assessment of autoregulation, we could not reliably go above the ambient blood pressure so the ability to assess higher and lower pressures with the in vitro approach compliment the in vivo data.

Another inherent issue associated with direct comparison of the in vitro data with the in vivo data is the analysis. In vitro data were analyzed based on the actual juxtamedullary afferent arteriole diameter and changes in diameter normalized to the control or starting diameter. Diameter responses were analyzed in response to increases in renal perfusion pressure up to 170 mmHg. The results indicate that the response in HS conditions was significantly shallower that the other groups and is most clearly manifested at the higher pressures. In the in vivo setting we measured whole kidney RBF responses emerging from decreases in RAP. Under these conditions changes in RBF reflect resistance changes in all of the renal vasculature in contrast to the diameter response of a single arteriole in a single region of the kidney. Because of the challenges inherent in using artificial means to increase RAP to approximately 170 mmHg in an anesthetized rat setting, we chose to assess autoregulatory responses by manipulating RAP from ambient to 85 mmHg. Each group of rats/conditions presented with slightly different ambient RAP and ET receptor blockade tended to reduce RAP. Consequently, the analysis was conducted from ambient RAP to 85 mmHg and
normalized accordingly. This approach yields what appears to be a discrepant autoregulatory profile with impairment apparent at the higher pressures in the in vitro studies and at lower pressures in the in vivo studies. The end conclusion is that autoregulatory capability is impaired under HS conditions and that it is improved by inhibition of the ET system. The directionality of whether it is at higher or lower pressures in this report is a function of the normalization and analysis.

Consistent with our in vivo findings and our previous report (15), Saeed et. al. reported that high dietary salt impairs dynamic autoregulation of RBF in rats infused with angiotensin II (55). They employed transfer function analysis to evaluate the renal myogenic response and the tubuloglomerular feedback response and found that both were significantly impaired and that the impairment could be improved by treatment with tempol. More recently, they extended those studies by assessing the impact of endothelin receptor blockade on the impaired dynamic autoregulation using an internally paired design similar to that used for our studies (54). In addition, the treatment doses of the endothelin receptor antagonists were identical to those used in the current study. In contrast to our finding that joint administration of ET_A/ET_B receptor blockers or a selective ET_B blocker alone markedly improved autoregulatory function, they found that endothelin receptor antagonists did not attenuate the abnormalities in dynamic autoregulation observed in their angiotensin II-infused, HS model. Indeed, Kittikulsuth et al demonstrated that ET_B receptor activity is decreased in Ang II-infused hypertension in male rats but not female rats (34). The reason for this discrepancy could relate to the use of a more complex experimental setting including high dietary salt and angiotensin...
II-induced hypertension. Both of these conditions independently have a negative impact on autoregulatory control but the mechanisms responsible for each are not understood.

Increased ET-1 production in HS conditions and impairment of renal microvascular autoregulatory efficiency could represent a physiological response of the kidney to excrete more salt. Reduced autoregulatory efficiency would facilitate subtle increases in glomerular filtration pressure to increase the filtered sodium load. Under such conditions, increased delivery of sodium to the collecting duct would exacerbate ET<sub>B</sub> receptor dependent inhibition of tubular sodium reabsorption that occurs during HS intake, thereby maintaining sodium balance. Consequently, upregulation of the renal and vascular endothelin system may represent an important renal response to high dietary salt to facilitate excretion of the salt load and maintain cardiovascular homeostasis.

In summary, this study provides compelling new evidence for the hypothesis that increased salt intake leads to an endothelin receptor-dependent decrease in afferent arteriole autoregulatory behavior. This impaired autoregulatory function related to a HS appears to involve ET<sub>B</sub> receptors and may also involve ET<sub>A</sub> receptors or a complex ET<sub>A</sub>/ET<sub>B</sub> receptor interaction. These observations could enhance the therapeutic potential of endothelin receptor blockers in the prevention of kidney injury due to excess salt consumption and perhaps salt-sensitive hypertension. It is interesting to speculate that in a setting of high dietary salt, that endothelin signaling may be linked to increased
oxidative stress previously shown to blunt autoregulatory behavior under high dietary salt conditions (15; 16; 55).
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References


**Figure Legends**

**Figure 1.** Baseline afferent arteriolar diameter at a perfusion pressure of 100 mmHg using the in vitro juxtamedullary nephron technique, from rats fed 0.8%NaCl (solid fill) or 8.0%NaCl (cross hatched) before and during exposure to the ETA receptor blocker, BQ-123 (white), or ETB receptor blocker, BQ-788 (gray). Receptor blockers were administered in the blood perfusate. Data are expressed as the mean ± SE; n = the number of afferent arterioles studied. * P < 0.05 vs. NS.

**Figure 2.** Afferent arteriolar diameter response to changing perfusion pressure in kidneys using the in vitro juxtamedullary nephron technique. A: depicts the response of 0.8%NaCl fed rats (black symbols) and 8.0%NaCl fed rats (white symbols) with BQ-123 (squares) and without BQ-123 (circles). BQ-123 was administered in the blood perfusate. B: data expressed as a percent of the control diameter. Autoregulatory profile ranges from 65 to 170 mmHg. Data are expressed as the mean ± SE; n = the number of afferent arterioles studied. * P < 0.05 between HS vs. NS. # P < 0.05 between HS vs. HS+BQ-123.

**Figure 3.** Afferent arteriolar diameter response to changing perfusion pressure in kidneys using the in vitro juxtamedullary nephron technique. A: depicts the response of 0.8%NaCl fed rats (black symbols) and 8.0%NaCl fed rats (white symbols) with BQ-788 (diamonds) and without BQ-788 (circles). BQ-123 was administered in the blood perfusate. B: data expressed as a percent of the control diameter. Autoregulatory profile ranges from 65 to 170 mmHg. The data plotted here for the NS and HS groups
are the same data plotted from Figure 2. They are included here for comparison. Data are expressed as the mean ± SE; n = the number of afferent arterioles studied. * P < 0.05 between HS vs. NS. # P < 0.05 between HS+ BQ-788.

**Figure 4.** RBF response to decreasing renal arterial pressure in kidneys of NS (0.8% NaCl) and HS (8.0%NaCl) fed rats before and after combined ETA (BQ-123, 30nmoles/kg/min) and ETB (BQ-788, 30nmoles/kg/min) receptor blockade. A: illustrates actual RBF responses for NS and HS rats before and after administration of BQ-123/BQ-788. B: presents data normalized to baseline RBF. Autoregulation was studied by adjusting left kidney RAP from ambient to 105, 95 and 85 mmHg. Data are expressed as the mean ± SE; n = the number of rats studied. * P < 0.05 between HS vs. NS. # P < 0.05 between HS + BQ-123/BQ-788.

**Figure 5.** Autoregulatory Index (AI) for RBF when decreasing renal arterial pressure in kidneys of NS (0.8% NaCl) and HS (8.0%NaCl). AI is presented for RBF when pressure is reduced from ambient to 105 mmHg (Left Panel) and from ambient to 95 mmHg (Right Panel) calculated during endothelin receptor blockade or in the absence of receptor blockers for the NS and HS alone groups. Data are expressed as the mean ± SE; n = the number of rats (8 for all groups) studied. * P < 0.05 significant difference compared to NS. # P < 0.05 significant difference compared to HS.

**Figure 6.** RBF response to decreasing renal arterial pressure in kidneys of HS (8.0%NaCl) fed rats before and during ETA receptor blockade with BQ-123 (Panels A
and B, 30nmoles/kg/min) or during ETB receptor blockade with BQ-788 (Panels C and D; 30nmoles/kg/min). Panels A and C illustrate the actual RBF responses for HS rats before and during endothelin receptor blockade. Panels B and D present the data normalized to baseline renal blood flow. Autoregulation was studied by adjusting left kidney RAP from ambient to 105, 95 and 85 mmHg. Data are expressed as means ± SE; * P < 0.05 for HS-preBQ-123 infusion vs. HS + BQ-123.

Figure 7. The change in carotid artery pressure (MAP) in response to a bolus injection of ET-1 in HS treated rats. ET-1 (10 nmoles) was injected (i.v.) at the end of each protocol to assess the level of endothelin receptor blockade in rats fed HS diets or the vehicle (ETOH) control. Data are expressed as the mean change ± SE; * P < 0.05 from the pre-injection carotid artery pressure.

Figure 8. RBF response to decreasing renal arterial pressure in kidneys from ETB receptor deficient rats and Tg control rats fed NS or HS diets. Autoregulatory responses for Tg controls + NS (black circles), Tg controls +HS (gray circles), ETB-def + NS (black squares) and ETB-def + HS (gray squares). Data are presented as actual RBF responses as left kidney RAP was adjusted from ambient to 105, 95 and 85 mmHg. Data are expressed as means ± SE; * the number of rats studied. # indicates P<0.05 vs. WT+NS at the same MAP level. * indicates P<0.05 vs. the basal diameter in the same group. The horizontal error bars are within the symbols.
Figure 1

Afferent Arteriole Diameter (μm)

NS
Con
BQ123
BQ788

HS
Con
BQ123
BQ788

(n=6) (n=6) (n=6) (n=8) (n=6) (n=6)
Figure 3

A

Perfusion Pressure (mmHg)

Afferent Arteriolar Diameter (μm)

NS; n=6
HS; n=8
NS + BQ788; n=6
HS + BQ788; n=6

B

% of Control Diameter

NS; n=6
HS; n=8
NS + BQ788; n=6
HS + BQ788; n=6

Perfusion Pressure (mmHg)
Figure 4

A

Renal Arterial Pressure (mmHg)

RBF (ml/min)

NS Baseline; n=8
HS Baseline; n=8
NS + BQ123/BQ788; n=8
HS + BQ123/BQ788; n=8

B

% of Baseline RBF

NS Baseline; n=8
HS Baseline; n=8
NS + BQ123/BQ788; n=8
HS + BQ123/BQ788; n=8

Renal Arterial Pressure (mmHg)
Figure 5

Ambient MAP to 105 mmHg

Ambient MAP to 95 mmHg

Autoregulatory Index

-0.4  -0.2  0.0  0.2  0.4  0.6  0.8  1.0  1.2

NS  HS  NS-BQ123/BQ788  HS-BQ123/BQ788  HS-EtOH  HS-BQ123  HS-BQ788

*  #  *  #  #

NS  HS  NS-BQ123/BQ788  HS-BQ123/BQ788  HS-EtOH  HS-BQ123  HS-BQ788

*  #  #  *  #  #
Figure 6

A

B

C

D

Renal Arterial Pressure (mmHg)

80 90 100 110 120 130

RBF (ml/min)

6 8 10 12 14

% of Baseline RBF

60 70 80 90 100 110

Renal Arterial Pressure (mmHg)

80 90 100 110 120 130

Renal Arterial Pressure (mmHg)

80 90 100 110 120 130

Renal Arterial Pressure (mmHg)

80 90 100 110 120 130

HS pre/ BQ123; n=8

HS w/ BQ123; n=8

HS pre/ BQ788; n=8

HS w/ BQ788; n=8

HS pre/ BQ123; n=8

HS w/ BQ123; n=8

HS pre/ BQ788; n=8

HS w/ BQ788; n=8

*
Figure 7

[Bar chart showing change in MAP (mmHg) for different treatments: Vehicle, BQ123/BQ788, BQ123, and BQ788. The chart indicates statistical significance with an asterisk (*) and non-significance (NS).]
Figure 8

Renal Arterial Pressure (mmHg)

RBF (ml/min)

WT+NS; (n=8)
ETB+NS; (n=6)
WT+HS; (n=8)
ETB+HS; (n=7)

* # #