Blockade of Distal Nephron Sodium Transport Attenuates Pressure Natriuresis in Dogs

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Abstract The sodium excretory responses (UNV) to acute changes in renal arterial pressure (RAP) during blockade of distal nephron sodium transport were evaluated in seven sodium-replete anesthetized dogs. The major distal sodium entry pathways were blocked by intrarenal infusion of amiloride (AM, 10^-5 mol/L) and bendroflumethiazide (BZ, 10^-4 mol/L). Infusion of AM plus BZ caused slight increases in renal blood flow (RBF, 4.1±0.5 to 4.6±0.4 mL.min^-1.g^-1; P<.001) but no changes in glomerular filtration rate (GFR, 0.96±0.05 to 1.01±0.07 mL.min^-1.g^-1; P=NS) or autoregulatory efficiency of RBF and GFR. There were significant increases in UNV (2.7±0.7 to 5.2±0.6 µmol.min^-1.g^-1) and fractional excretion of sodium (FENa, 1.8±0.4% to 3.5±0.3%) and decreases in potassium excretion (0.35±0.06 µmol.min^-1.g^-1) during AM plus BZ infusion. During the control period and during repeat measurements in time control studies, decreases in RAP (150 to 100 mm Hg) elicited the usual decreases in UNV (slope, 0.022±0.007 µmol.min^-1.g^-1 mm Hg^-1; P<.01). After administration of AM plus BZ, there was a marked attenuation of the pressure-natriuretic responses, and the slopes of the RAP versus UNV and RAP versus FENa relations at RAP levels above 100 mm Hg were not significantly different from zero. However, the pressure-natriuresis response was maintained at arterial pressure between 75 and 100 mm Hg. Addition of the nitric oxide (NO) synthesis inhibitor, nitro-L-arginine (NLA, 50 µg.kg^-1.min^-1) during AM plus BZ infusion resulted in decreases in RBF to 3.79±0.34 mL.min^-1.g^-1, UNV to 2.89±0.16 µmol.min^-1.g^-1, and FENa to 2.13±0.09% without appreciable changes in GFR. The pressure-natriuretic responses remained attenuated during NLA infusion. These data suggest that the sodium entry pathways in the distal nephron are involved in mediating the arterial pressure-induced changes in sodium excretion occurring at RAPs above 100 mm Hg. The reduced sodium excretion caused by NLA during distal nephron blockade suggests that NO influences additional sodium excretory mechanisms. (Hypertension. 1994;23[part 2]:1040-1045.)

Key words • amiloride • bendroflumethiazide • natriuresis • nitric oxide • nitro-L-arginine • sodium channels

Although it has long been recognized that the kidney has the ability to alter urinary sodium excretion (UNV) in response to acute changes in renal arterial pressure (RAP), the mechanisms mediating pressure natriuresis remain unclear. As this phenomenon of pressure natriuresis can be observed during autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR), it is generally agreed that alterations in tubular sodium reabsorption rather than changes in filtered load are primarily responsible for the pressure-dependent changes in sodium excretion. However, the tubular segments involved in this mechanism remain uncertain. Some studies have suggested a major role for proximal tubular segments either collectively or primarily in the deep nephrons, whereas others have indicated that the distal tubule or the collecting duct segments, or both, may be involved in mediating this pressure-natriuretic response.

Recent evidence indicates that intrarenal nitric oxide (NO) exerts a substantive role in regulating UNV as well as in mediating pressure natriuresis. The exact mechanism of NO-induced changes in tubular transport of sodium is not yet clearly defined. Although some of the natriuretic actions of NO may be due to associated intrarenal hemodynamic changes, recent in vitro studies performed in cultured cortical collecting duct cells indicate that NO may also exert a direct effect on epithelial transport mechanisms via an amiloride (AM)-sensitive pathway. Collectively, these studies have suggested that distal nephron sodium entry pathways may participate in mediating pressure natriuresis. The present investigation was designed to examine the possible role of distal nephron sodium entry pathways in mediating pressure natriuresis and the extent to which NO blockade may overlap with these mechanisms. At least two separate pathways mediate sodium transport across the luminal membrane of cells in distal nephron segments. One pathway is the electron-neutral-coupled sodium chloride cotransport pathway, which can be blocked by the thiazide diuretics. The other pathway is a conductive channel and can be blocked by AM. Thiazide diuretics are known to inhibit apical membrane sodium chloride cotransporter in the distal and collecting duct nephron segments. Among the thiazide agents, bendroflumethiazide (BZ) has the least inhibitory action on carbonic anhydrase. AM has been widely used as a distal-acting, potassium-sparing diuretic. At low doses (10^-5 mol/L), this drug acts by blocking sodium channels in the luminal cell membrane resulting in a reduction of net sodium transport across the distal tubular epithelium.

Because blocking only one of these pathways might augment transport through the other, the rationale of these experiments was to achieve an effective blockade of distal sodium entry by the combined intrarenal administration of AM plus BZ. The UNV responses to acute changes in RAP were evaluated before and during
combined AM plus BZ infusion. In addition, NO synthesis inhibition was superimposed to determine if the antinatriuretic effects of NO blockade persisted during distal sodium transport blockade.

**Methods**

Experiments were carried out in 10 mongrel dogs (19.6±1.1 kg body wt) of either sex. The preparation of the animals and basic experimental techniques are similar to those previously described. To achieve a sodium-replete state, the dogs were given supplemental amounts of sodium chloride (1.5 g/kg body wt per day for 3 days) added to the normal laboratory diet. The dogs were anesthetized with pentobarbital sodium (30 mg/kg body wt IV) and artificially ventilated. Body temperature was maintained within the normal range (99° to 101°F) using an electric heating pad. Systemic arterial pressure (AP) of these dogs was measured from a catheter placed in the abdominal aorta inserted via the right femoral artery. The catheter was connected to a pressure transducer, and AP was recorded on a polygraph (model 7D, Grass Instruments). The left femoral artery was cannulated for collection of blood samples. The femoral and jugular veins were cannulated for administration of an insulin solution and additional doses of pentobarbital sodium as necessary. Normal isotonic saline was infused into the right femoral vein at a rate of 30 mL/h during the entire experimental period.

The left kidney was exposed through a flank incision and was denervated by cutting the renal nerves. The rationale for renal denervation was to minimize the effects of increased sympathetic activity in the kidney after carotid occlusion. RBF was measured with an electromagnetic flow probe, which was placed on the renal artery and connected to a square wave flowmeter (Carolina Medical Electronics). An adjustable plastic clamp was placed around the renal artery distal to the flow probe to achieve reductions in RAP. A curved 23-gauge needle cannula was inserted into the renal artery distal to the plastic clamp and connected to another pressure transducer to measure RAP. Another catheter was also connected to this needle cannula for continuous infusion of heparinized saline or drug solutions at a rate of 0.4 mL/min. Urine was collected from a catheter placed in the ureter.

After completion of all surgical procedures, a 2.5% solution of inulin in normal saline was administered. A priming dose of 1.6 mL/kg body wt of inulin solution was followed by a sustaining infusion of 0.03 mL/min/kg body wt. The right common carotid artery was occluded and the left common carotid artery was partially constricted to elevate the basal level of AP to about 150 mm Hg. This procedure was conducted at least 1 hour before the onset of the experimental protocol. Forty-five minutes after the initiation of inulin infusion the experimental protocol started with urine collections for two consecutive 10-minute periods at spontaneous RAP. At the midpoint of each urine collection period, an arterial blood sample (2 mL) was taken to measure plasma inulin, sodium, and potassium concentrations. Then step reductions in RAP (125, 100, and 75 mm Hg) were produced by adjusting the clamp. At each level of RAP, 5 minutes were allowed for stabilization before a 1-minute urine sample was collected. Below 75 mm Hg of AP, RAP was further reduced in steps of 15 to 20 mm Hg for 2 to 3 minutes until RBF was reduced to near zero. After the last reduction in RAP, the clamp was released completely to reestablish control RAP and RBF. After control measurements were taken in seven dogs, a continuous intrarenal infusion of BZ (0.28 μg·kg\(^{-1}\)·min\(^{-1}\); Sigma Chemical Co.) was initiated. This dose of BZ was used to achieve an estimated approximate concentration of 10\(^{-3}\) mol/L in the renal arterial plasma. Ten minutes after the initiation of the combined AM plus BZ infusion, the previous protocol was repeated to examine the renal responses to reductions in RAP. Following reestablishment of the control RAP and RBF at the end of the experimental protocol with the AM plus BZ infusion, nitro-L-arginine (NLA, 50 μg·kg\(^{-1}\)·min\(^{-1}\); Aldrich Chemical Co, Inc) was added to the AM plus BZ infusion. Thirty minutes after the initiation of NLA infusion, the protocol was again repeated to examine the renal responses to reductions in RAP in the presence of both tubular luminal Na\(^+\) entry blockade and NO synthesis inhibition.

During combined infusion of AM plus BZ in the renal artery, there were small but statistically significant decreases in AP and in RVR. RBF increased without a change in GFR. Urine flow, U\(_{\text{NaV}}\), fractional excretion of sodium (FE\(_{\text{Na}}\)), and urinary potassium excretion (U\(_{\text{K}}\)) increased substantially during BZ infusion alone. The urinary concentration of sodium did not change significantly (225±18 to 208±21 mmol/L). The mean urine osmolality (851±156 to 544±130 mosmol/L) fell slightly but remained hypotonic.

**Results**

**Effect of BZ and AM Infusion on Renal Hemodynamics and Function**

The Table summarizes the results obtained in seven dogs. Control systemic AP was elevated to 158±1 mm Hg due to the partial constriction of the carotid arteries. BZ infusion alone did not cause any significant change in basal level of AP, renal vascular resistance (RVR), RBF, or GFR. However, urine flow, U\(_{\text{NaV}}\), fractional excretion of sodium (FE\(_{\text{Na}}\)), and urinary potassium excretion (U\(_{\text{K}}\)) increased substantially during BZ infusion alone. The urinary concentration of sodium did not change significantly (225±18 to 208±21 mmol/L). The mean urine osmolality (851±156 to 544±130 mosmol/L) fell slightly but remained hypotonic.

During combined infusion of AM plus BZ in the renal artery, there were small but statistically significant decreases in AP and in RVR. RBF increased without a change in GFR. U\(_{\text{NaV}}\) and FE\(_{\text{Na}}\) increased further but U\(_{\text{NaV}}\) decreased significantly. Urinary concentration of sodium also increased significantly to 294±20 mmol/L. However, urine osmolality did not change significantly (600±130 mosmol/L).

**Effect of AM Plus BZ Infusion on Renal Autoregulation**

Fig 1 illustrates the effect of combined infusion of AM plus BZ on autoregulatory efficiency of RBF and GFR. The autoregulation plateau of RBF was slightly elevated due to a small increase in basal level of RBF during combined infusion of AM plus BZ. However, there were...
no changes in the slopes of the autoregulatory portion (−0.003±0.002 to −0.005±0.006 mL·min⁻¹·mm Hg⁻¹) or the linear portion (0.07±0.007 to 0.08±0.007 mL·min⁻¹·mm Hg⁻¹) of the RBF autoregulation curve (Fig 1A) during AM plus BZ infusion. The autoregulatory efficiency of GFR (Fig 1B) at mean RAP above 75 mm Hg remained intact during both control and combined infusion of AM plus BZ periods.

Effect of AM plus BZ Infusion on the Renal Excretory Responses to Reductions in RAP

Fig 2 illustrates the effects of AM plus BZ infusion on pressure-induced changes in the excretory function of the kidney. Although sodium entry blockade by AM plus BZ infusion caused markedly increased urine flow and sodium excretion, the slopes of the relations between AP and either urine flow (not shown) or sodium excretion (Fig 2) were markedly attenuated at APs above 100 mm Hg during AM plus BZ infusion. The autoregulatory efficiency of GFR (Fig 1B) at mean RAP above 75 mm Hg remained intact during both control and combined infusion of AM plus BZ periods.

As shown in the Table, addition of NLA (50 μg·kg⁻¹·min⁻¹) to the intrarenal AM plus BZ infusion line for more than 30 minutes resulted in an increase of 21±5% in RVR and decreases of 17±2% in RBF, 48±6% in urine flow, 42±5% in UN,V, and 37±5% in FE Na values, without any significant change in GFR (−7±3%) and UN,KV (−1±6%). The overall relative decreases in urine flow, UN,V, and FE Na values during NLA administration in these AM plus BZ-treated dogs were less than what was observed previously in our laboratory in untreated salt-repleted dogs.10,11

![Fig 1](http://hyper.ahajournals.org/)

**Fig 1.** Renal blood flow (RBF) (A) and glomerular filtration rate (GFR) (B) responses to acute reductions in renal arterial pressure (RAP) before (○) and during (△) combined intrarenal infusion of amiloride (AM) and bendroflumethiazide (BZ) and during (●) addition of nitro-L-arginine (NLA) with AM plus BZ infusion in anesthetized dogs (n=7). Mean curves for blood flow autoregulation were generated by extrapolating the values of RBF at different levels of RAP (at 25-mm Hg intervals ranging from 150 to 25 mm Hg) from each individual curve obtained in each dog. Two separate linear regression analyses of the pressure-flow relations were carried out in each dog to obtain the extrapolated values at the pressure levels at which RBF was auto-regulated and at lower pressure levels at which a linear relation between RAP and RBF was obtained. Error bars indicate SEM.
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Fig 2. Sodium excretion (UN,V) (A) and fractional excretion of sodium (FENa) (B) responses to acute changes in renal arterial pressure (RAP) above 75 mm Hg before (o) and during (A) amiloride (AM) plus bendroflumethiazide (BZ) infusion and during (•) combined infusion of AM plus BZ plus nitro-L-arginine (NLA) (n=7). The responses to reductions in RAP during the control period were attenuated during AM plus BZ infusion and remained persistently attenuated during AM plus BZ plus NLA infusion at RAP above 100 mm Hg.

Fig 1 illustrates the effect of NLA infusion on autoregulatory efficiency of RBF and GFR in these AM plus BZ-treated dogs. Similar to our earlier reported observations the basic pattern of autoregulatory efficiency of RBF and GFR remained intact during NLA infusion, but the RBF autoregulation plateau was lowered.

After addition of NLA, the values of urine flow, UN, and FENa at spontaneous levels of RAP decreased from the values during combined AM plus BZ infusion (Table). The excretory responses to reductions in RAP above 100 mm Hg remained attenuated during addition of NLA (Fig 2) as reported earlier. The slopes of the relation between RAP versus UN and RAP versus FENa at the first and second periods were 0.03±0.004 μmol·min⁻¹·g⁻¹·mm Hg⁻¹ and 0.02±0.006 to 0.02±0.003%·mm Hg⁻¹, respectively. After the second period, when saline infusion rates were doubled from 30 mL·h⁻¹ to 60 mL·h⁻¹, there was an augmentation of the pressure-induced natriuretic response. The slopes of the relation between RAP versus UN and RAP versus FENa were increased to 0.045±0.013 μmol·min⁻¹·g⁻¹·mm Hg⁻¹ and 0.02±0.008%·mm Hg⁻¹ during saline load. The changes in RBF, GFR, UN,V, and FENa were 5.55±0.94 mL·min⁻¹·g⁻¹, 1.22±0.02 mL·min⁻¹·g⁻¹, 5.13±1.0 μmol·min⁻¹·g⁻¹, and 2.87±0.58%, respectively.

**Discussion**

As expected, the intrarenal administration of BZ (0.28 μg·min⁻¹·kg⁻¹) and AM (1.7 μg·min⁻¹·kg⁻¹) resulted in substantial increases in UN,V as well as FENa (Table and Fig 2). These increases in UN,V were not associated with significant changes in GFR. The doses of AM as

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**Effects of Time and Volume Expansion on Renal Excretory Responses to Reduction in RAP**

Fig 3 reports results obtained in the absence of pharmacological interventions to determine time-dependent effects on the pressure-induced natriuretic responses in three dogs. During the first and second control periods, there were no perceptible changes in RBF (4.39±0.55 to 4.83±0.89 mL·min⁻¹·g⁻¹), GFR (1.01±0.04 to 1.06±0.06 mL·min⁻¹·g⁻¹), UN,V (3.21±0.61 to 3.10±0.33 μmol·min⁻¹·g⁻¹), and FENa (2.19±0.49% to 1.99±0.25%). Also there were no significant changes in the pressure-natriuretic response between the first and the second period. The slopes of the RAP versus UN and RAP versus FENa relation during the first and second periods were 0.03±0.004 μmol·min⁻¹·g⁻¹·mm Hg⁻¹ and 0.02±0.006 to 0.02±0.003%·mm Hg⁻¹, respectively. After the second period, when saline infusion rates were doubled from 30 mL·h⁻¹ to 60 mL·h⁻¹, there was an augmentation of the pressure-induced natriuretic response. The slopes of the relation between RAP versus UN and RAP versus FENa were increased to 0.045±0.013 μmol·min⁻¹·g⁻¹·mm Hg⁻¹ and 0.02±0.008%·mm Hg⁻¹ during saline load. The changes in RBF, GFR, UN,V, and FENa were 5.55±0.94 mL·min⁻¹·g⁻¹, 1.22±0.02 mL·min⁻¹·g⁻¹, 5.13±1.0 μmol·min⁻¹·g⁻¹, and 2.87±0.58%, respectively.
well as BZ were selected to achieve an effective blockade of sodium entry pathways in the distal nephron segments without greatly influencing proximal transport mechanisms. The results indicate that inhibition of sodium entry pathways in the distal nephron segments by AM plus BZ infusion greatly attenuate the excretory responsiveness to changes in AP above 100 mm Hg. Such an attenuation of the pressure-natriuresis relation was not due to time-dependent deterioration of the experimental preparations since it was observed that the slope of this relation did not change significantly over the same time period for AM plus BZ infusion in control animals (Fig 3); also, the renal blood flow and GFR were preserved within normal range during AM plus BZ and NLA infusion (Table) and autoregulatory behavior was not altered (Fig 1).

The attenuation of the steep part of the pressure-natriuresis curve above RAP of 100 mm Hg during AM plus BZ infusion suggests that the sodium entry pathways in the distal nephron segment are primarily responsible for mediating the excretory responses to changes in RAP at the higher APs. It should be emphasized that this lack of a pressure-natriuresis response during a high level of sodium excretion is unusual and not simply because sodium excretion is greatly increased. Acute saline loading, which caused similar increases in urinary sodium excretion, resulted in an augmentation of these pressure-induced natriuretic responses (Fig 3). Indeed, a variety of agents that increase sodium excretion such as atrial natriuretic peptide (ANP), angiotensin converting enzyme inhibitors, and other vasodilators markedly increase, not decrease, the slope of the pressure-natriuresis relation. It may be argued that the diuretic agents (AM plus BZ) blocked the pressure-natriuretic relation because they increase distal delivery of sodium secondary to washout of the medullary gradient. However, saline loading (Fig 3) and ANP administration, which would cause similar effects on the medullary gradient, increased the slope of the pressure-induced natriuretic responses. At the doses used, AM plus BZ infusion appears to have its major effects by inhibition of distal nephron sodium transport. At higher doses, proximal Na+/H+ exchange and carbonic anhydrase activity may be inhibited, but when this occurs there is often a tubuloglomerular feedback–mediated decrease in GFR. Nevertheless, this study cannot rule out other possible actions of these agents on renal function, and we do not mean to imply that the changes in distal sodium transport are solely responsible for this pressure-natriuretic response.

It is interesting that the sodium excretion responses to changes in RAP during AM plus BZ are markedly different from those observed during ANP infusion, since it has been shown that ANP can inhibit sodium transport via an AM-sensitive sodium channel. However, ANP also exerts hemodynamic effects. AM plus BZ pharmacologically inhibits the tubular sodium channels, whereas ANP exerts an inhibitory effect on distal sodium transport as a consequence of increased cell cyclic GMP. Presumably, other regulatory mechanisms can continue to interact at the cellular level to influence transport rate.

Previous micropuncture studies in rats have reported some contrasting results about the role of the medullary collecting duct in pressure natriuresis. Sonnenberg et al reported an inhibition of the sodium chloride reabsorption in the medullary collecting duct during increased perfusion pressure, whereas Roman observed an increase in reabsorption of chloride and water in the papillary collecting duct segments. The present study is consistent with elevations in AP resulting in inhibition of distal nephron sodium transport primarily at APs above 100 mm Hg. The cause of the decrease in sodium excretion rate at 75 mm Hg RAP during AM plus BZ infusion despite no appreciable change in the filtered load is not clear, but this finding supports the concept that multiple mechanisms are responsible for pressure natriuresis. It is possible that there was concomitant augmentation of the renin-angiotensin system at the lower AP. Another possibility is that UaV at this lower range of RAP may be influenced by other mechanisms such as peritubular physical factors involving decreases in interstitial intrastatic pressure or changes in medullary hemodynamics that may have a predominant role at pressures in the lower autoregulatory range.

Intrarenal administration of NLA during combined infusion of AM plus BZ lowered RBF and sodium excretion and there was a maintained attenuation of the pressure-natriuretic curve. As shown in the Table, there were reductions in absolute and fractional excretion of sodium during NO synthesis inhibition in the AM plus BZ–treated dogs. Since it has been suggested that NO mediates sodium transport by modulating the activity of an AM-sensitive sodium channel, NO blockade might be expected to be without an effect during pharmacological blockade. The finding that sodium excretion still decreased suggests the effects of NO on distal sodium transport were not completely blocked by the doses of AM plus BZ or that NO influences tubular sodium reabsorption via other mechanisms as well. Nevertheless, the relative decrease in sodium excretion was much less than what has been observed previously in untreated salt-repleted dogs in our laboratory. Thus, these in vivo findings are in partial agreement with in vitro studies that the NO-induced changes in UaV are due, at least in part, to its direct inhibitory action on the sodium channels in tubular epithelium. The residual decrease in UaV during NLA infusion in AM plus BZ–treated dogs observed in the present study may be due to the hemodynamic changes observed during NO synthesis inhibition. It is possible that NO-induced hemodynamic changes, either cortical or medullary, indirectly influence tubular reabsorptive rate in other nephron segments. Further experiments are needed to delineate the relative contributions of hemodynamics and tubular factors to the sodium excretory responses after inhibition of NO synthesis.

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References


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