Determinants of Renal Actions of Atrial Natriuretic Peptide

Lack of Effect of Atrial Natriuretic Peptide on Pressure-Induced Vasoconstriction

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We have previously demonstrated that atrial natriuretic peptide (ANP) completely reverses norepinephrine-induced afferent arteriolar (AA) vasoconstriction. In the present study we characterized the effects of ANP on pressure-induced vasoconstriction of AA. Chronic unilateral hydrenephrosis was induced to facilitate direct visualization of the renal microcirculation. Hydrenephrotic kidneys were perfused in vitro, and AA diameters were measured during stepwise alterations in renal arterial pressure. Increasing renal arterial pressure from 80 to 180 mm Hg decreased AA diameter by 22±2% (from 18.5±1.0 to 14.4±1.0 μm, p<0.005). In the presence of 100 nM ANP [human ANP-(4–28)], AA vasoconstricted by 23±4%, indicating that ANP failed to modify the pressure-induced AA vasoconstriction. Furthermore, both nitroprusside (10 μM) and 8-bromoguanosine 3′:5′-cyclic monophosphate (30 μM) only partially inhibited pressure-induced AA vasoconstriction (31±5% and 47±7%, respectively), whereas these vasodilators completely abolished norepinephrine-induced AA vasoconstriction. In contrast, nifedipine completely inhibited pressure-induced AA vasoconstriction. In summary, pressure-induced AA vasoconstriction is insensitive to the action of ANP, is relatively refractory to cyclic GMP-mediated vasorelaxation, but is completely inhibited by calcium channel blockade. Furthermore, since ANP completely abolishes norepinephrine-induced vasoconstriction but fails to affect pressure-induced vasoconstriction, it is apparent that the type of underlying vasoconstrictor stimuli constitutes a major determinant of the renal microvascular response to ANP. (Circulation Research 1990;67:1–10)

Atrial natriuretic peptide (ANP) is a potent vasodilator hormone. It has recently been discovered that ANP binds to a unique receptor possessing guanylate cyclase activity at an intracellular domain.1 Thus, activation of ANP receptor leads to an elevation of guanosine 3′:5′-cyclic monophosphate (cGMP) as the intracellular mediator of ANP-induced vasorelaxation.2–4 It has been reported that ANP is a renal-selective vasodilator.5,6 Indeed, ANP has been demonstrated to dilate the afferent arteriole in a variety of experimental settings.7–9 Conversely, it has also been suggested that renal microvessels are insensitive to ANP.10 Furthermore, in contrast to other vasodila-

tors,11,12 ANP exerts little effect on renal blood flow when administered in vivo.13,14 It is thus readily apparent that the renal microvascular actions of ANP have not been fully delineated. Furthermore, the determinants of the renal microvascular responsiveness to ANP have not been defined.

We have recently demonstrated that pregglomerular microvessels vasoconstrict markedly in response to an elevation in renal perfusion pressure.15 Although the relative roles of tubuloglomerular feedback and pressure-induced vasoconstriction remain controversial, myogenic vasoconstriction of pregglomerular vessels is considered to contribute in part to renal autoregulation.16,17 In vivo studies indicate that, whereas renal autoregulation is impaired by diverse vasodilators including calcium antagonists, prostaglandins, and dopamine,11,12 it is not altered by ANP.18,19 Although such observations suggest that ANP does not alter the renal hemodynamic responses to pressure, the effects of ANP on pressure-induced afferent arteriolar vasoconstriction have not been directly ascertained.

In the present study, we directly assessed the effects of ANP and the cGMP-related vasodilators, nitroprus-
side and 8-bromoguanosine 3′:5′-cyclic monophosphate (8-bromo-cGMP), on the afferent arteriolar vasoconstriction induced by increased renal perfusion pressure. Our findings indicate that pressure-induced afferent arteriolar vasoconstriction is insensitive to the actions of ANP and relatively refractory to cGMP-dependent vasodilation, whereas it is completely inhibited by calcium antagonists. In concert with our previous observation that ANP completely reverses norepinephrine-induced afferent arteriolar vasoconstriction,7 the results of the present study demonstrate the dominant influence of underlying vasoconstrictor stimuli as a major determinant of the subsequent renal hemodynamic response to this peptide hormone.

Materials and Methods

Hydronephrotic kidneys from 6-week-old male Sprague-Dawley rats were used for in vitro perfusion studies. The right ureter was ligated under methoxyflurane anesthesia. After 8–10 weeks of chronic unilateral hydronephrosis, tubular atrophy of the right kidney had progressed to a stage that allowed direct microscopic visualization of the renal microvessels.20 The hydronephrotic kidneys were excised and perfused in vitro as briefly described below, and as detailed in previous communications.7,15,21

The animals were anesthetized with 5-sec-butyl-5-ethyl-thiobarbituric acid (100 mg/kg Inactin, Byk-Gulden, Constance, FRG). The renal artery of the hydronephrotic kidney was cannulated in situ and perfused continuously with warm oxygenated media. The perfusate consisted of a Krebs-Ringer bicarbonate buffer containing 5 mM d-glucose, 7.5% bovine serum albumin (Bovuminar, Armour Pharmaceutical, Kankakee, Ill.), and a complement of amino acids as described previously.22 The hydronephrotic kidney was then excised and perfused on the stage of an inverted microscope (model K, Nikon, Tokyo, Japan) modified to accommodate a heated chamber equipped with a thin glass viewing port on the bottom surface. Kidneys were allowed to equilibrate for at least 30 minutes before initiating the experimental protocol.

The apparatus that was used to study the hydronephrotic kidneys in vitro is depicted in our previous publication.7 The perfusion media was saturated with a gas mixture of 95% O2-5% CO2 within a pressurized reservoir. The perfusion pressure was monitored at the level of the renal artery and controlled by a back-pressure-type regulator (model 10BP, Fairchild Industrial Products, Winston-Salem, N.C.). Perfusion flow was monitored by means of an extracorporeal electromagnetic flow probe (model 300A, Carolina Medical Electronics, King, N.C.) placed in the perfusion circuit immediately proximal to the kidney.

Segments of afferent arterioles between its origin from the interlobular artery and the midregion were selected for quantitation of the response.15 Vessel diameters were measured as detailed in a previous publication.7,15,21 In brief, video images were digitized and analyzed with automated software. Vessel segments approximately 50 μm in length were analyzed at 2–5-second intervals. Diameters were estimated from the mean distance between parallel edges of the selected vascular segment. Mean vessel diameters were obtained by averaging all determinations obtained during the period of measurement. Each value of vessel diameter thus obtained was a mean of at least 30 individual measurements.

The ability of ANP and cGMP-related vasodilators (nitroprusside and 8-bromo-cGMP) or of a calcium antagonist (nifedipine) to prevent pressure-induced afferent arteriolar vasoconstriction was ascertained by measuring changes in vessel diameter before and after the addition of each agent to the perfusing medium. Renal microvessels were observed as renal arterial pressure (RAP) was elevated stepwise by 20–mm Hg increments from a basal value of 80 mm Hg to a maximum of 180 mm Hg. The diameters of the afferent arterioles were determined for at least 1 minute at each level of RAP.

After the observations of the basal pressure–induced afferent arteriolar vasoconstriction, ANP (100 nM, n=6), nitroprusside (1 and 10 μM, n=5), 8-bromo-cGMP (10 and 30 μM, n=5), or increasing concentrations of nifedipine (1 nM to 10 μM, n=6) were added to the perfusate. The vasoconstrictor response of the same vessel segment was then reassessed as described above.

The ability of ANP to reverse pressure-induced vasoconstriction was also determined in six additional hydronephrotic kidney preparations. In these experiments, RAP was increased from 80 to 180 mm Hg to induce afferent arteriolar vasoconstriction. ANP (100 nM) was then added to the perfusate. After observing the afferent arteriolar response to ANP, RAP was returned to 80 mm Hg. Thereafter, the ability of the same vessel segment to respond to diltiazem was determined, following the same protocol.

To compare the inhibition by nitroprusside or 8-bromo-cGMP of pressure-induced afferent arteriolar vasoconstriction with that of norepinephrine-induced vasoconstriction, additional experiments were conducted. Hydronephrotic kidneys were perfused at a constant pressure of 80 mm Hg. After pretreatment with propranolol (3 μM), 0.3 μM norepinephrine was administered to the perfusate. Subsequently, increasing doses of nitroprusside (10 nM, 100 nM, 1 μM, and 10 μM, n=6) or 8-bromo-cGMP (10 μM and 30 μM, n=6) were added to reverse norepinephrine-induced afferent arteriolar vasoconstriction.

All studies were conducted using a yellow filter in the fiberoptic illuminator to avoid photo-induced degradation of the dihydropyridine. The ANP that was used in the present study was the 4–28 amino acid sequence of human atrial peptide (Ayerst Wyeth Laboratories, Philadelphia). Nifedipine and diltiazem were gifts from Pfizer Laboratories, New York, and Marion Laboratories, Kansas City, Mo., respectively. Nitroprusside and 8-bromo-cGMP were obtained from Sigma Chemical, St. Louis. All stock solutions were prepared on the day of study. Polyethylene glycol was used as the vehicle for nifedipine while other substances were dissolved in
deionized water. At the maximal concentrations employed (0.1%), the vehicle alone had no effect on the renal microvessels.15,21

All data are expressed as mean±SEM. Data were analyzed by one-way analysis of variance followed by Student’s t test. Changes within experimental groups were subjected to paired analysis; differences between groups were assessed by unpaired analysis. Values of p<0.05 were considered statistically significant.

Results

Effects of ANP

Figure 1 depicts tracings of afferent arteriolar diameters from a representative study in which pressure-induced vasoconstriction was assessed before and after the administration of 100 nM ANP. ANP did not alter the pressure-induced response of this vessel. Thus, in the absence of ANP, the afferent arteriolar diameter decreased from 20.1 μm (at 80 mm Hg) to a minimal diameter of 15.2 μm (at 180 mm Hg) as RAP was increased in a stepwise manner. In the presence of 100 nM ANP, the response of the afferent arteriole was similar (i.e., decreasing from 20.9 to 16.4 μm as pressure was increased from 80 to 180 mm Hg).

Figure 2 summarizes the effects of ANP (100 nM) on pressure-induced afferent arteriolar vasoconstriction examined in six hydronephrotic kidney preparations. In the absence of ANP, increasing RAP induced graded reductions in afferent arteriolar diameters. In comparison to basal diameter (18.5±1.0 μm at 80 mm Hg, n=6), a significant vasoconstriction was observed at 100 mm Hg (17.9±1.0 μm, p<0.05), and further reductions in diameter were noted as RAP was raised to 160 mm Hg. The elevation in RAP from 160 to 180 mm Hg elicited no further afferent arteriolar vasoconstriction (14.8±0.9 μm at 160 mm Hg vs. 14.4±1.0 μm at 180 mm Hg, p>0.1).

In the presence of ANP (100 nM), the basal diameter (18.5±0.4 μm at 80 mm Hg) was identical to that observed in the absence of ANP (p>0.5). Furthermore, as RAP was elevated, afferent arteriolar diameter decreased in a manner similar to that observed in the absence of the peptide. Thus, a significant reduction in afferent arteriolar diameter was observed at 100 mm Hg (16.8±0.6 μm, p<0.025). At each level of RAP, afferent arteriolar diameter in the presence of ANP did not differ significantly from that observed in the absence of the peptide (p>0.1 or greater). Thus, ANP failed to prevent or modify pressure-induced afferent arteriolar vasoconstriction at a concentration that we had previously found to completely reverse norepinephrine-induced vasoconstriction of this vessel.7

The results presented above indicate that ANP is ineffective in preventing pressure-induced afferent arteriolar vasoconstriction. To further assess the effects of ANP on pressure-induced vasoconstriction, we examined the ability of this peptide to reverse this vasoconstrictor response when administered to vessels precontracted by elevated pressure. Figure 3 depicts a tracing from a representative study. As can be seen, ANP produced very little effect on afferent arteriolar diameter when the peptide was administered during pressure-induced vasoconstriction (Figure 3, left). In contrast, when the calcium antagonist diltiazem (10 μM) was administered to this same vessel under identical conditions, the pressure-induced vasoconstriction was completely reversed. Actually, the elevated RAP produced a passive distension of the afferent arteriole in the presence of diltiazem (Figure 3, right).

Studies in six afferent arterioles disclosed that increasing RAP from 80 to 180 mm Hg decreased afferent arteriolar diameter from 18.8±0.6 to 13.7±0.6 μm (p<0.001). The administration of ANP (100 nM) pro-
Effects of cGMP-Related Vasodilators

The vasodilatory actions of ANP are mediated, in large part, by an elevation of cGMP induced by activation of particulate guanylate cyclase.2-4 Therefore, we examined the inhibition of pressure-induced afferent arteriolar vasoconstriction by nitroprusside and 8-bromo-cGMP. To assess the effectiveness of nitroprusside and 8-bromo-cGMP in this model, we also determined the effects of these vasodilators on norepinephrine-induced afferent arteriolar vasoconstriction.

Figure 4, left panel, depicts a representative tracing illustrating the inhibition by nitroprusside of norepinephrine-induced afferent arteriolar vasoconstriction. Norepinephrine (0.3 µM) decreased vessel diameter from 20.3 to 15.5 µm. Nitroprusside reversed norepinephrine-induced afferent arteriolar vasoconstriction in a dose-dependent manner. Thus, 10 µM nitroprusside completely returned afferent arteriolar diameter to the control level (20.7 µm). Subsequent administration of phentolamine (10 µM) did not increase the vessel diameter further (20.5 µm). When the mean responses of afferent arterioles from six hydronephrotic kidneys were assessed, norepinephrine decreased afferent arteriolar diameter by 27±2% (i.e., from 19.2±0.5 to 14.0±0.6 µm, p<0.001). Nitroprusside (1 µM) returned the afferent arteriolar diameter to 17.6±0.5 µm (p<0.001), which corresponded to 69±5% reversal from the norepinephrine-induced afferent arteriolar vasoconstriction. Nitroprusside (10 µM) completely reversed the vasoconstriction of this vessel (i.e., 19.2±0.5 µm [nitroprusside] vs. 19.2±0.5 µm [control], p>0.5).

Similarly, 8-bromo-cGMP was also potent in inhibiting norepinephrine-induced vasoconstriction of the afferent arteriole (Figure 4, right panel). Norepinephrine decreased afferent arteriolar diameter from 20.4 to 14.3 µm. The subsequent addition of 10 µM 8-bromo-cGMP dilated the afferent arteriole to 16.3 µm. Further administration of 30 µM 8-bromo-cGMP restored the vessel diameter to 18.9 µm. In six afferent arterioles, norepinephrine decreased vessel diameters from 19.3±0.4 to 15.0±0.3 µm (p<0.001). At 10 µM, 8-bromo-cGMP reversed norepinephrine-induced afferent arteriolar vasoconstriction by 60±13% (i.e., to 17.5±0.5 µm, p<0.01), and 30 µM 8-bromo-cGMP completely returned the afferent arteriolar diameter to the control level (18.8±0.3 µm, p>0.1).

In contrast to the potent actions of nitroprusside and 8-bromo-cGMP on norepinephrine-induced afferent arteriolar vasoconstriction, both substances...
exhibited diminished vasodilatory effects when the vessel was activated by pressure. Figure 5 summarizes the inhibition by nitroprusside (1 and 10 μM) of the pressure-induced afferent arteriolar vasoconstriction in five hydropnephrotic kidneys. At 1 μM, nitroprusside had no effect on the pressure-induced vasoconstriction (closed squares). A modest, albeit significant, inhibition was observed at 10 μM nitroprusside (closed triangles). Results are mean±SEM (n=5).

Figure 5. Graph showing effects of nitroprusside on pressure-induced afferent arteriolar (AA) vasoconstriction. At 1 μM, nitroprusside had no effect on the pressure-induced vasoconstriction (closed squares). A modest, albeit significant, inhibition was observed at 10 μM nitroprusside (closed triangles). Results are mean±SEM (n=5).

Figure 6. Graph showing effects of 8-bromoguanosine 3'5'-cyclic monophosphate (8-bromo-cGMP) on pressure-induced afferent arteriolar (AA) vasoconstriction. At 10 μM, 8-bromo-cGMP had no effect on pressure-induced vasoconstriction (closed squares). A partial inhibition of pressure-induced vasoconstriction (i.e., 160 and 180 mm Hg) was observed at 30 μM 8-bromo-cGMP (closed triangles). Results are mean±SEM (n=5).

Vasodilation induced by ANP was not affected by 8-bromo-cGMP at either 10 or 30 μM. However, 8-bromo-cGMP did partially inhibit nitroprusside-induced vasoconstriction (Figure 6). At 10 and 30 μM, 8-bromo-cGMP had no effect on the basal (i.e., at 80 mm Hg) afferent arteriolar diameter (20.1±0.6 μm at control, 19.8±0.6 μm at 10 μM 8-bromo-cGMP, and 20.1±0.8 μm at 30 μM 8-bromo-cGMP; p>0.5, n=5). Furthermore, at 10 μM, 8-bromo-cGMP did not affect the vasoconstrictor response to pressure. Thus, increasing RAP from 80 to 180 mm Hg evoked the identical afferent arteriolar vasoconstriction (−24±4% [10 μM 8-bromo-cGMP] vs. −27±5% [control], p>0.5). At 30 μM, 8-bromo-cGMP only partially inhibited the pressure-induced vasoconstriction; significant vasoconstriction was observed at 100 mm Hg (19.6±0.9 μm at 100 mm Hg vs. 20.1±0.8 μm at 80 mm Hg, p<0.05), and further elevation in RAP to 180 mm Hg decreased the vessel diameter to 16.9±0.8 μm (p<0.01 vs. 20.1±0.8 μm at 80 mm Hg), corresponding to a 16±2% decrement from the basal diameter. Thus, 30 μM 8-bromo-cGMP inhibited pressure-induced afferent arteriolar vasoconstriction elicited by the increase in RAP from 80 to 180 mm Hg by only 47±7%.

Figure 6. Graph showing effects of 8-bromoguanosine 3'5'-cyclic monophosphate (8-bromo-cGMP) on pressure-induced afferent arteriolar (AA) vasoconstriction. At 10 μM, 8-bromo-cGMP had no effect on pressure-induced vasoconstriction (closed squares). A partial inhibition of pressure-induced vasoconstriction (i.e., 160 and 180 mm Hg) was observed at 30 μM 8-bromo-cGMP (closed triangles). Results are mean±SEM (n=5).

Effects of Calcium Antagonists

In contrast to the lack of vasodilatory effect of ANP and the modest inhibition by nitroprusside and 8-bromo-cGMP, nifedipine completely abolished pressure-induced vasoconstriction of the afferent arteriole. In the representative study illustrated in Figure 7, increasing RAP from 80 to 180 mm Hg decreased afferent arteriolar diameter from 18.2 to 12.8 μm. After administration of 1 μM nifedipine, the pressure-induced vasoconstriction of the afferent arteriole was inhibited. Indeed as RAP was increased, the afferent arteriole appeared to be passively dilated by the higher pressure (i.e., 19.8 μm at 180 mm Hg).

Figure 7 summarizes dose-response data obtained from six kidneys in which the effects of nifedipine (1 nM to 10 μM) on the pressure-induced afferent arteriolar response to pressure were ascertained. In the absence of nifedipine (open circles in Figure 8), a significant afferent arteriolar vasoconstriction was observed at 100 mm Hg (16.4±0.8 μm at 100 mm Hg)
FIGURE 7. Representative tracings demonstrating the complete inhibition by nifedipine of pressure-induced afferent arteriolar vasoconstriction. Stepwise elevations in renal arterial pressure elicited a marked vasoconstriction in the absence of nifedipine (left). In contrast, increasing renal arterial pressure tended to dilate this vessel in the presence of nifedipine (10⁻⁵ M) (right).

vs. 19.3±1.2 µm at 80 mm Hg, n=6, p<0.05). At 180 mm Hg, afferent arteriolar diameter was reduced to 12.8±0.7 µm (p<0.001).

Nifedipine caused a dose-dependent inhibition of pressure-induced vasoconstriction of the afferent arteriole. In the presence of nifedipine, the basal afferent arteriolar diameter (i.e., at 80 mm Hg) was identical to that of control (19.3±1.2 µm) at concentrations up to 1 µM nifedipine (19.1±1.2 µm at 1 nM, 19.2±1.3 µm at 10 nM, 19.8±1.0 µm at 100 nM, and 20.4±1.1 µm at 1 µM; p>0.05, n=6). The decrease in afferent arteriolar diameter elicited by increasing RAP was inhibited by nifedipine in a dose-dependent manner (Figure 8). At 1 µM, nifedipine completely inhibited pressure-induced activation of the afferent arteriole (p>0.1). At 10 µM nifedipine, the basal diameter was significantly greater (i.e., 21.4±1.3 µm, p<0.05 vs. control), and elevation of RAP tended to increase, rather than decrease, afferent arteriolar diameter (i.e., 22.5±1.6 µm at 180 mm Hg vs. 21.4±1.3 µm at 80 mm Hg).

FIGURE 8. Graph of mean data summarizing the inhibition by nifedipine of pressure-induced afferent arteriolar vasoconstriction. Nifedipine inhibited this response in a dose-dependent fashion (closed symbols). Results are mean±SEM (n=6).

FIGURE 9. Graph comparing the dose dependency for the reversal by nifedipine of pressure-induced (i.e., 180 mm Hg) afferent arteriolar vasoconstriction (closed circles, n=6) with that subtending the reversal of KCl-induced (30 nM) afferent arteriolar vasoconstriction (open circles, n=8). The IC₅₀s for nifedipine to inhibit pressure- (157±65 nM) and KCl-induced vasoconstriction (34±18 nM) were similar (p>0.1). The data for KCl are adapted in part from Reference 21. Results are mean±SEM.

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side; \( p > 0.2 \) with 10 \( \mu M \) nitroprusside). Similarly, 10 \( \mu M \) 8-bromo-cGMP had no effect on pressure-induced alterations in RVR (\( p > 0.05 \)), although 30 \( \mu M \) 8-bromo-cGMP prevented the increase of RVR associated with the increase in pressure (1±1% [30 \( \mu M \) 8-bromo-cGMP] vs. 11±2% [control], \( p < 0.001 \)). Nevertheless, in striking contrast to the results obtained with nifedipine (see below), elevated pressure was not associated with a decrease in RVR in the presence of 30 \( \mu M \) 8-bromo-cGMP.

In contrast to these vasodilators, nifedipine elicited marked effects on the RVR response to pressure. After administration of 10 nM nifedipine, the increase in RVR associated with the elevation in RAP was completely abolished (i.e., from 8.6±2.0 to 8.6±2.0 mm Hg/min/ml, \( p > 0.2 \)). At higher concentrations of nifedipine, elevation in RAP from 80 to 180 mm Hg resulted in a marked reduction in RVR (−12±6% at 100 nM nifedipine, −19±4% at 1 \( \mu M \) nifedipine, and −22±3% at 10 \( \mu M \) nifedipine). Thus, the administration of the calcium antagonist unmasked a pressure-induced decrement in RVR that was probably due to a passive distension of the renal vasculature.

In summary, although the isolated perfused hydronephrotic kidney does not manifest complete auto-regulation, RVR increases in response to elevated perfusion pressure. More importantly, we have demonstrated that nifedipine administration unmasks a pressure-induced reduction in RVR (Figure 10).

This observation suggests that the effects of pressure-induced afferent arteriolar vasoconstriction on RVR in this in vitro model are underestimated due to a concomitant countervailing decrease in RVR, possibly mediated by passive distension of larger vessels. Regardless, our observations demonstrate that both ANP and nitroprusside had no effect and that 8-bromo-cGMP only partially attenuated the increase in RVR associated with the elevation in RAP, an observation that is in accordance with the effects of these agents on pressure-induced afferent arteriolar vasoconstriction.

**Discussion**

Despite extensive study, the effects of ANP on the renal microvasculature have not been definitively characterized.5–9,13,14,24,25 ANP has been reported to either increase5,6,13,14 or exert little effect13,14 on renal blood flow and glomerular filtration rate in vivo. We have demonstrated that the microcirculation of the isolated perfused hydronephrotic kidney model is highly responsive to ANP when renal vascular tone is established by norepinephrine.7 Conceivably, the underlying renal vasoconstrictor stimuli may constitute important determinants of the renal microvascular response to ANP. Recently we have demonstrated that the isolated perfused hydronephrotic kidney constitutes an appropriate model for assessing the renal microvascular response to elevated pressure.15 The present study was undertaken to ascertain if the renal microcirculation responds to ANP during renal vasoconstriction induced by elevated pressure per se.

Our present results demonstrate conclusively that even at superphysiological concentrations, ANP does not inhibit pressure-induced vasoconstriction of the afferent arteriole. Similarly, another vasodilator known to act through cGMP, nitroprusside, and 8-bromo-cGMP were also relatively ineffective in inhibiting pressure-induced vasoconstriction. In striking contrast, we have previously demonstrated in other strains15 that calcium antagonists completely abolish pressure-induced vasoconstriction in this model.

**Effects of ANP on the Renal Circulation**

In addition to the discrepant reports of the actions of ANP on renal blood flow and glomerular filtration rate, controversy also attends the effects of ANP on the renal microcirculation. Edwards and Weidley10 reported that ANP did not dilate the afferent arteriole microdissected from rabbit kidneys during norepinephrine-induced activation of this vessel. In contrast, we demonstrated complete afferent arteriolar vasodilation in the isolated perfused hydronephrotic rat kidneys during norepinephrine-induced vasoconstriction.7 Similarly, in a preliminary report, Yuan et al16 observed that ANP reversed the norepinephrine-induced vasoconstriction of the afferent arteriole microdissected from rat kidneys. Furthermore, Marin-Grez et al17 and Veldkamp et al18 demonstrated that ANP dilated afferent arterioles in vivo.
hydromelectic kidneys and in vitro rat juxta-
medullary nephrons, respectively. In contrast, Lappe
et al. \(^{27}\) demonstrated that ANP failed to increase
renal blood flow in the conscious rat. Finally, ANP
does not impair renal autoregulation in either the
rat\(^{18}\) or dog.\(^ {19}\) These seemingly discrepant observa-
tions suggest that the ability of ANP to dilate the
renal vasculature varies depending on the experi-
mental settings, including animal species, models,
and types of vasoconstrictor stimuli.

In the present study, we have demonstrated that
ANP does not dilate the afferent arterioles when
these vessels are activated by elevated pressure (Fig-
ures 1, 2, and 3). Similarly, Steinhausen et al.\(^ {28}\) noted
that in the in vivo hydromelectic rat, even in the
presence of maximally effective doses of ANP, a
reduction of perfusion pressure elicited a further
afferent arteriolar vasodilation. These investiga-
tors inferred, albeit indirectly, that pressure-dependent
vasoconstriction was insensitive to ANP. In the
present study, we marshal direct and conclusive evidence
that ANP neither prevents (Figures 1 and 2) nor
reverses (Figure 3) pressure-induced vasoconstric-
tion of the afferent arteriole. Furthermore, our
observations were obtained in an in vitro setting in
which renal vascular tone was determined exclusively
by pressure,\(^ {15}\) precluding possible confounding
effects. In concert with our previous demonstration,
in the identical model, that ANP completely reverses
norepinephrine-induced afferent arteriolar vasoconstric-
tion,\(^ {2}\) the present findings offer compelling evi-
dence that an underlying vasoconstrictor stimulus
constitutes a major determinant of the renal micro-
vascular response to ANP.

**Effects of Nitroprusside and 8-Bromo-cGMP on
Pressure-Induced Vasoconstriction**

ANP is a cGMP-dependent vasodilator,\(^ {2-4}\) and the
ANP receptor has recently been identified as a unique
form of guanylate cyclase.\(^ {1}\) cGMP also mediates
the vasodilator actions of nitroprusside.\(^ {29}\) In the present
study, we have demonstrated that both nitroprusside
(10 \(\mu\)M) and 8-bromo-cGMP (30 \(\mu\)M) only partially
inhibit pressure-induced afferent arteriolar vasoconstric-
tion (31\(\pm\)5% and 47\(\pm\)7%, respectively) at concen-
trations that completely reverse norepinephrine-
induced afferent arteriolar vasoconstriction. In
concert with our current demonstration that ANP
fails to alter pressure-induced vasoconstriction, these
findings indicate that pressure-induced afferent arte-
riolar vasoconstriction is resistant to cGMP-mediated
vasodilation.

The observation that ANP was totally ineffective,
whereas nitroprusside and 8-bromo-cGMP were par-
tially effective, in preventing pressure-induced vaso-
constriction may be ascribed to different levels of
cGMP attained. Alternatively, these different re-
sponses may be due to differences in the subcellular
compartmental distribution of cGMP, since ANP
activates the particulate form\(^ {4}\) and nitroprusside
stimulates the soluble form of guanylate cyclase.\(^ {29}\)

Since cGMP alters vasoconstrictor responses by
diverse mechanisms,\(^ {30-33}\) such quantitative and qual-
itative differences may account for the differing
effects of these cGMP-dependent vasodilators. Fur-
thermore, we observed that ANP induced a transient,
modest vasoconstriction of vessels preconstricted by
 elevated pressure (Figure 3). It is possible that such high
levels of ANP (10 \(\mu\)M) may cause a transient
increase in the rate of cGMP generation that exceeds
steady-state levels.

**Divergent Effects of cGMP-Mediated Vasodilators
on Receptor-Mediated Versus
Depolarization-Induced Activation**

It has been reported that vascular smooth muscle
responds differently to ANP and cGMP-mediated
vasodilators when the preparations are preconstric-
ted with differing vasoconstrictors.\(^ {3,33,34}\) ANP
effectively relaxes the \(\alpha\)-adrenergic– angiotensin
II–stimulated aortic strips.\(^ {24,33-35}\) In contrast,
contractions elicited by KCl-induced depolarization are
refractory to ANP,\(^ {33,34}\) although submaximal KCl-
induced contractions have been reported to be inhib-
ited by ANP.\(^ {33,34}\) Furthermore, ANP has no effect on
potassium-induced calcium influx, although it effec-
tively inhibits norepinephrine-stimulated calcium
influx.\(^ {35}\) Similarly, nitroprusside inhibits norepine-
phrine-induced contractions, but not KCl-induced con-
tractions of canine renal arterial strips.\(^ {36}\) Taken
together, these divergent effects of ANP and nitro-
prusside suggest that cGMP-mediated vasodilators
selectively inhibit receptor-mediated vasoconstric-
tion, whereas depolarization-induced vasoconstric-
tion is refractory to cGMP.\(^ {3}\)

We have previously reported that ANP completely
reverses norepinephrine-induced afferent arteriolar vasoconstriction.\(^ {7}\) In the present study, we observed
that nitroprusside and 8-bromo-cGMP also com-
pletely reverse norepinephrine-induced vasoconstric-
tion of this vessel (Figure 4). In striking contrast,
ANP, nitroprusside, and 8-bromo-cGMP were ineffec-
tive in inhibiting pressure-induced afferent arteri-
olar vasoconstriction. In concert with the formulation
that pressure-induced vasoconstriction is mediated
by depolarization-induced activation of voltage-
dependent calcium channels (see below), our present
findings are consistent with the premise that cGMP-
mediated vasodilators selectively inhibit receptor-
mediated, but not depolarization-induced, vasocon-
striction of the afferent arteriole.

**Mechanisms of Pressure-Induced Vasoconstriction**

The mechanisms mediating pressure-induced affer-
ent arteriolar vasoconstriction have not been fully elu-
cidated. Several lines of evidence indicate that
pressure-induced vasoconstriction may be mediated by
a pressure-induced membrane depolarization. Harder
et al.\(^ {23}\) demonstrated that elevated pressure elicits
membrane depolarization in isolated canine interlobu-
lar arteries and that verapamil prevented the pressure-
induced vasoconstriction of this vessel. In the present
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study, we observed that nifedipine and diltiazem abolished the afferent arteriolar response to elevated pressure. We have previously reported similar observations in kidneys of Wistar-Kyoto and spontaneously hypertensive rats. Furthermore, as depicted in Figure 9, the dose dependency for inhibition by nifedipine of pressure-induced vasoconstriction of the afferent arteriole was similar to that previously reported to reverse KCl-induced vasoconstriction of this vessel. In concert, these findings support the postulate that pressure elevation may activate voltage-dependent calcium channels, thereby eliciting vasoconstriction in the afferent arteriole.

Based on these considerations, one may inquire why ANP affects solely norepinephrine-induced vasoconstriction, whereas calcium antagonists abolish both pressure- and norepinephrine-induced afferent arteriolar vasoconstriction. Figure 11 summarizes a theoretical schema reconciling these seemingly discrepant observations. We propose that both norepinephrine and elevated pressure activate voltage-dependent calcium channels in the afferent arteriole through membrane depolarization. We suggest, however, that norepinephrine and pressure activate such calcium channels by differing mechanisms. Since ANP has been demonstrated to interfere with receptor-linked activating mechanisms in vascular smooth muscle, it may disrupt the coupling between receptor occupation and calcium channel activation. In contrast, elevated pressure may induce membrane depolarization through a more direct mechanism. Such formulation would explain why receptor-mediated (i.e., norepinephrine) activation of calcium channels is abolished by ANP, whereas pressure-mediated activation of calcium channels is not.

The striking dependency of ANP's actions on underlying basal renal vascular tone may account for seemingly discrepant observations on the renal hemodynamic effects of ANP in diverse settings. Furthermore, modified renal responsiveness to ANP in pathophysiological settings may not necessarily reflect alterations in ANP-linked vasodilatory mechanisms but, rather, may result from intrinsic or extrinsic factors affecting renal vasoconstrictor mechanisms.

Effects of Vasodilators on RVR

In the present study, a close concordance was observed between the effects of vasodilators on pressure-induced afferent arteriolar vasoconstriction and changes in RVR. Thus, elevating RAP caused a significant increase in RVR, which was not altered by ANP or nitroprusside (Figure 10). Furthermore, 8-bromo-cGMP did not prevent the attendant rise in RVR at 10 M, although at 30 M 8-bromo-cGMP, RVR did not increase in response to elevation of RAP. In striking contrast, in the presence of nifedipine elevation of RAP resulted in a marked decrease in RVR.

Although elevated RAP produced striking vasoconstriction of the afferent arteriole, the attendant increase in RVR appeared to be attenuated. The administration of nifedipine, however, revealed a concurrent pressure-induced reduction in RVR when the afferent arteriolar vasoconstriction was inhibited. Thus, the tendency for elevated pressure to passively decrease RVR was countered by the afferent arteriolar vasoconstriction, the latter causing a significant increase in RVR.

Implications of the Present Observations for the In Vivo Renal Hemodynamic Effects of ANP

Recent investigations indicate that autoregulation of renal blood flow is maintained intact during ANP administration. Paul et al demonstrated that atriopeptin II and ANP did not alter the autoregulation of renal blood flow and glomerular filtration rate in response to acute changes in blood pressure. Similarly, Lappe et al also observed that ANP had no effect on renal autoregulation in rats in vivo. Finally, Steinhausen et al reported that in the presence of ANP, glomerular blood flow was maintained constant during alterations in renal perfusion pressure. In the present study, we have demonstrated conclusively that ANP fails to abolish the afferent arteriolar vasoconstriction elicited by the elevation of renal arterial pressure. Our present findings, therefore, are consistent with the above-cited in vivo observations.

Our observations may explain why ANP does not perturb renal autoregulation but has been demonstrated to disrupt tubuloglomerular feedback. Thus, in the present study, the direct vasoconstrictor response of the afferent arteriole to elevated pressure is preserved in the presence of ANP. We suggest, therefore, that this mechanism may account for the intact autoregulatory response observed in the presence of ANP.

In conclusion, our findings indicate that the underlying vasoconstrictor stimulus of the afferent arteriole constitutes the major determinant of the response of this vessel to ANP. In this regard, the vasodilator profile of ANP reflects the differing sensitivities of vasoconstrictor mechanisms to cGMP. In concert with recent observations from this laboratory,
we propose that cGMP alters receptor-mediated afferent arteriolar vasoconstriction but has little effect on depolarization-induced vasoconstriction. This formulation explains why ANP alters norepinephrine-induced but not pressure-induced afferent arteriolar vasoconstriction.

References


Key Words: atrial natriuretic peptide • renal microcirculation • renal vascular resistance • calcium antagonists • renal autoregulation • nitroprusside • cyclic GMP
Determinants of renal actions of atrial natriuretic peptide. Lack of effect of atrial natriuretic peptide on pressure-induced vasoconstriction.

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*Circ Res.* 1990;67:1-10
doi: 10.1161/01.RES.67.1.1

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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