SUMMARY We examined the consequences of genetic susceptibility or resistance to NaCl-induced hypertension and of prior salt loading (high or low NaCl intake) on the responses of isolated perfused Dahl salt-sensitive (DS) and Dahl salt-resistant rat (DR) kidneys to atriopeptin II. Atriopeptin II increased the glomerular filtration rate only in kidneys from high NaCl-fed rats, irrespective of their DS or DR status. Superimposition of norepinephrine on atriopeptin II further increased the glomerular filtration rate only in kidneys from low NaCl-fed rats (which had not reacted to atriopeptin II alone), irrespective of their DS or DR status, and did not change the glomerular filtration rate of high NaCl-fed rats. Norepinephrine alone, without atriopeptin II, uniformly decreased the glomerular filtration rate by about 80%. Atriopeptin II increased sodium excretion of high NaCl and low NaCl DR kidneys by more than five times as much as in the corresponding DS kidneys. Therefore, the glomerular filtration rate response to atriopeptin II varied globally with dietary NaCl, independently of genetic predisposition or resistance to NaCl-induced hypertension. The natriuretic response to atriopeptin II was blunted in kidneys from rats genetically susceptible to NaCl-induced hypertension, independently of their NaCl consumption. Atriopeptin II also ameliorated or reversed the adverse effect of norepinephrine on the glomerular filtration rate. (Hypertension 11:745–749, 1988)

KEY WORDS • Dahl rats • atrial natriuretic factor • atriopeptin II • isolated perfused rat kidney • norepinephrine vasoconstriction • salt-induced hypertension
12 ± 1 weeks of age (mean ± SEM) and weighed 342 ± 9 g. The DR were on average 13 ± 1 weeks of age and weighed 382 ± 12 g. Rats were anesthetized with Inactin (100 mg/kg i.p.), and the mean arterial blood pressure was measured directly in the external carotid or femoral artery using a pressure transducer (Harvard Instruments, Millis, MA, USA).

Our kidney perfusion procedure entails the removal of the kidney from the rat without ischemia and has been reported previously.11 Perfusion was done at 37°C using a recirculating solution containing predialyzed bovine serum albumin (BSA Fraction V, Pentex-Miles, Kankakee, IL, USA) at a concentration of 6.5 to 7 g/dl. The perfusate contained electrolytes, glucose, inulin, and amino acids at concentrations reported previously.11 Equilibration with 95% O2, 5% CO2 resulted in a perfusate pH of 7.40 to 7.42. The perfuser initially was passed through a 0.45-μm filter (Amicon, Lexington, MA, USA), and a 5-μm filter (Nucleopore, Pleasanton, CA, USA) was incorporated into the circuit.

Perfusate flow was measured with a Brooks flowmeter (Type R2-15B Thomas Scientific, Philadelphia, PA, USA). The hydraulic pressure of the perfusion system was monitored in order to maintain the renal perfusion pressure constant at 105 mm Hg. This value took into account the resistance imposed by the needle cannulating the renal artery. After an initial 20- to 30-minute equilibration period, urine and perfusate specimens were obtained for two control periods of 5 minutes each. Synthetic rat atriopeptin II (AP II; Sigma Chemical) then was added to the perfusate at a concentration of 60 ng/ml. After allowing 5 minutes for the stabilization of renal vascular resistance (RVR), specimens were obtained for two control periods of 5 minutes each. Sufficient norepinephrine (Parke-Davis) then was superimposed on the AP II treatment to increase RVR by 50% over control. This change required mean norepinephrine concentrations ranging from 44 to 53 ng/ml, values that did not differ significantly among the four groups (p = 0.185).

The high and low NaCl kidney groups differed markedly in their GFR reactivity to AP II treatment (Figure 2). AP II treatment increased the GFR of the DR/Hi kidneys by 474 ± 81 μl/min. Likewise, AP II treatment increased the GFR of DS/Hi kidneys by 400 ± 46 μl/min, an increase not significantly different from the DR/Hi group (p = 0.492). On the other hand, AP II treatment did not significantly increase the GFR of either the DR/Lo or the DS/Lo kidneys.

The superimposition of norepinephrine on AP II treatment increased the GFR of DR/Lo and DS/Lo kidney groups (see Figure 2), groups whose GFR had not responded to AP II alone. GFR showed a tendency to increase further with norepinephrine treatment in the two high NaCl groups, but the increases were not sig-

![Graph](attachment:image.png)

**FIGURE 1. Reactivity of DS and DR kidney renal vascular resistance (RVR) to atriopeptin II (AP II) before and after norepinephrine (NE) superimposition. Hi = high NaCl regimen; Lo = low NaCl regimen. Asterisks indicate significant changes (p<0.05) from the previous phase (i.e., to the left). AP II had variable effects on RVR. Numbers of experiments are in parentheses.**
Figure 2. Reactivity of Dahl rat kidney glomerular filtration rate (GFR) to atriopeptin II (AP II). AP II increased the GFR substantially only in high NaCl (Hi) kidneys irrespective of their DS or DR status. Norepinephrine (NE) superimposition increased the GFR only in low NaCl (Lo) kidneys (which had been hyporeactive to AP II alone) irrespective of their Dahl status.

Figure 3. Glomerular filtration rate (GFR) after norepinephrine (NE) alone, without atriopeptin II (AP II). Experiments were done over the same time frame as the studies with AP II. Hi = high NaCl diet; Lo = low NaCl diet.

Figure 4. Blunted atriopeptin II (AP II) natriuresis by DS kidneys, occurring independently of changes in glomerular filtration rate (see Figure 2). DS kidneys also manifested smaller changes in fractional sodium excretion (see text). Changes in sodium excretion were more strongly affected by Dahl status \((p = 0.001)\) than by prior NaCl loading \((p = 0.021)\). Hi = high NaCl diet; Lo = low NaCl diet; NE = norepinephrine.

Figurably larger than the values obtained with AP II treatment alone.

AP II pretreatment markedly affected the GFR responses of Dahl rat kidneys to norepinephrine. In separate control DS and DR high and low NaCl kidney groups, norepinephrine was added in amounts sufficient to increase RVR by 50%. In the absence of AP II, norepinephrine uniformly decreased the GFR (Figure 3). Decrements in GFR averaged \(377 \pm 60 \mu l/min\) for DS/Hi and \(248 \pm 63 \mu l/min\) for DR/Hi kidneys. The norepinephrine-induced decrement in GFR averaged \(202 \pm 36 \mu l/min\) for DS/Lo kidneys and \(275 \pm 54 \mu l/min\) for DR/Lo kidneys. Therefore, the presence of AP II eliminated or reversed adrenergically mediated decreases in GFR.

AP II pretreatment did not increase the absolute or fractional sodium excretion \((\text{FE}_{\text{Na}})\) in DS kidneys to the same extent as in DR kidneys at either dietary NaCl level (Figure 4). AP II increased absolute sodium excretion by \(7.4 \pm 1.7 \mu \text{Eq}/\text{min}\) in DR/Hi kidneys but only by \(1.3 \pm 0.4 \mu \text{Eq}/\text{min}\) in DS/Hi kidneys \((p = 0.016)\). AP II increased absolute sodium excretion by \(2.9 \pm 0.5 \pm 0.4 \mu \text{Eq}/\text{min}\) in DR/Lo kidneys and by \(0.34 \pm 0.11 \mu \text{Eq}/\text{min}\) in DS/Lo kidneys \((p = 0.019)\). Factoring by the GFR, \(\text{FE}_{\text{Na}}\) of DR/Hi kidneys increased from a control value of \(3.0 \pm 0.7\%\) to \(6.2 \pm 1.0\%\) after AP II treatment. \(\text{FE}_{\text{Na}}\) of DS/Hi kidneys increased from a control value of \(0.87 \pm 0.10\%\) to only \(1.56 \pm 0.29\%\) after AP II treatment. These changes in \(\text{FE}_{\text{Na}}\) differed significantly between the DS and DR kidneys \((p = 0.004)\). \(\text{FE}_{\text{Na}}\) of DR/Lo kidneys increased from a control value of \(4.98 \pm 0.82\%\) to \(9.11 \pm 1.2\%\) after AP II treatment, whereas \(\text{FE}_{\text{Na}}\) of DS/Lo kidneys increased from a control value of \(1.39 \pm 0.25\%\) to \(2.07 \pm 0.49\%\) after AP II treatment. The increase in \(\text{FE}_{\text{Na}}\) elicited by AP II was significantly greater for DR/Lo than for DS/Lo kidneys \((p = 0.001)\). In all four groups, the superimposition of norepinephrine on AP II treatment did not affect absolute sodium excretion significantly (see Figure 4). Norepinephrine superimposition did not change \(\text{FE}_{\text{Na}}\) of either of the high NaCl Dahl kidney groups, but it decreased DR/Lo kidney \(\text{FE}_{\text{Na}}\) significantly \((p < 0.001)\). In summary, AP II increased the GFR of DS/Hi and DR/Hi kidneys but had little effect on the GFR of DS/Lo and DR/Lo kidneys. However, the natriuretic response to AP II was blunted significantly in DS/Hi and DS/Lo kidneys as compared with DR/Hi and DR/Lo kidneys.

Discussion

The increased GFR elicited by ANF is accompanied by an augmented glomerular capillary pressure and ultrafiltration coefficient. The natriuretic action of ANF, while frequently attributable to hemodynamic alterations, can be ascribed to direct tubule transport inhibition in other situations with less prominent renal circulatory changes. Our results indicate that the action of ANF on the GFR is increased by NaCl load-
ing, irrespective of the presence or absence of NaCl-induced hypertension. According to other reports, renal and extrarenal circulatory actions of exogenous ANF may be enhanced by salt loading. In situations employing humans or animals, the possibility exists that the person or animal may be poised for enhanced reactivity to the peptide because of extracellular volume expansion or some circulatory alteration associated with a high NaCl intake. The present studies in isolated kidneys indicate that an NaCl-induced amplification of ANF action on the GFR can occur independently of extrarenal circulatory factors. Our data do not elucidate the mechanisms underlying the increased reactivity to ANF caused by salt. For example, a circulating substance released during NaCl loading could have been bound to the kidneys prior to their isolation and could have subsequently altered the renal response to AP II.

Under certain conditions, the numbers of vascular and mesangial ANF receptors in the rat change inversely with dietary NaCl. This occurrence may be a consequence of the down-regulation of ANF receptors by ANF itself. Other data indicate that renal ANF receptors and glomerular reactivity to ANF are not affected by NaCl loading. Although ANF receptors were not measured in our studies, the reports of an inverse relationship between receptor density and dietary NaCl raise the possibility that the increased glomerular reactivity to AP II following NaCl loading may not depend on alterations in receptor number. The high concentration of AP II used in our studies was chosen to elicit a maximum response and to make receptor saturation with ligand likely. Our use of a high AP II concentration and a different ANF molecular species may explain the differences between our results and those of others who failed to note an increased isolated kidney response to ANF after salt loading.

Because the intrarenal renin-angiotensin system is activated during salt restriction, renally generated angiotensin II may have antagonized the actions of AP II on kidneys from the low NaCl-fed rats despite the absence of angiotensinogen from the perfusate. However, the functional status of the intrarenal renin-angiotensin system within the isolated kidney is uncharacterized. The conversion of the preparation to a nonfiltering mode by elevation of the perfusate oncotic pressure with added albumin results in a brisk increase in renin release without any accompanying changes in RVR. In addition, the incorporation of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists into the perfusate does not increase the response of low NaCl kidneys to AP II, but instead appears to affect the AP II responses of high NaCl kidneys (T.H. Steele and L. Challoner-Hue, unpublished observations, 1987). Therefore, the possibility remains that operation of the intrarenal renin-angiotensin system may favor the generation of angiotensin II at sites critical for facilitating or opposing AP II action.

We have observed that isolated DS kidneys manifest increased RVR and GFR responses to a dihydropyri-

dine calcium channel agonist and antagonist, respectively. Differences between DS and DR kidney reactivity to these agents were accentuated by prior dietary NaCl loading. The present results indicate that the GFR responses to AP II differ from the responses to calcium channel agonists and antagonists, in that GFR reactivity to AP II varies globally with NaCl loading, independently of DS or DR status. Also, unlike calcium antagonists, AP II by itself can increase the GFR; calcium antagonists alone had no such effect on isolated kidneys. Similarly to calcium antagonists, AP II protected kidneys from functional deterioration during norepinephrine-induced vasconstriction. Finally, as with calcium antagonists, simultaneous treatment with AP II and norepinephrine increased the GFR of low NaCl DS and DR kidneys — organs that did not manifest a significant GFR response to AP II alone. This effect could be attributed to a selective preglomerular vasodilator action of AP II, either with or without a vasoconstrictor action on the postglomerular circulation.

AP II has been reported to inhibit the mobilization of calcium into the cytosol from intracellular storage sites in vascular smooth muscle. Other data suggest that this effect is specific for events originating through the activation of receptor-operated membrane calcium channels, as opposed to voltage-operated channels. However, AP II also has been reported to decrease resting cytosol calcium and to decrease cytosol calcium elevations following activation of either receptor-operated or voltage-operated calcium channels in vascular smooth muscle. These observations suggest that ANF may function in part as an endogenous calcium antagonist. It is not clear whether this phenomenon occurs secondarily to the activation of particulate guanylate cyclase by AP II or if it arises from some other property of AP II. Similarly to calcium antagonists, AP II was ineffective at eliciting a substantial natriuresis by the isolated DS kidney, even in the presence of very large increases in filtered sodium loads. Reduced natriuretic sensitivity to ANF by the DS kidney could be a factor underlying the elevated circulating immunoreactive ANF concentrations reported for the DS.

To the extent that they may be applicable to the intact animal, these results with isolated kidneys from a particular animal model of genetic susceptibility or resistance to the development of salt-induced hypertension suggest that glomerular responsiveness to ANF is accentuated by antecedent NaCl loading. This effect occurs independently of the presence or absence of a genetic predisposition to NaCl-induced hypertension. Natriuretic responsiveness to ANF, either in absolute terms or relative to the GFR, is blunted in the setting of genetic susceptibility to salt-induced hypertension — at least in the Dahl rat model. This refractoriness to ANF-induced natriuresis occurs independently of the antecedent dietary NaCl and is retained in the presence of large increases in the GFR. ANF also can prevent deterioration of renal function secondary to adrenergic stimulation, an attribute that may ultimately be ex-
ANF AND DAHL RAT KIDNEY/Steele and Challoner-Hue

exploited for the prevention or treatment of certain types of acute renal failure.29

References
Genetics and salt modulate renal responses to atrial natriuretic factor.
T H Steele and L Challoner-Hue

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