Genetics and Salt Modulate Renal Responses to Atrial Natriuretic Factor

THOMAS H. STEELE AND LAURA CHALLONE-HUE

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

Atrial natriuretic peptides, collectively known as atrial natriuretic factor (ANF), may be modulators of renal diuretic and natriuretic responses in salt-induced hypertension.1–3 We have observed that calcium channel antagonists can reverse the effects of norepinephrine vasoconstriction on isolated perfused rat kidneys by eliciting a large rebound increase in the glomerular filtration rate (GFR).4 This effect on GFR was accentuated in kidneys from normotensive Dahl salt-sensitive rats (DS), compared with kidneys from Dahl salt-resistant rats (DR).5 The difference in GFR response between DS and DR kidneys was amplified further after the DS rats exhibited NaCl-induced hypertension.6 In addition, renal vascular reactivity of DS kidneys to a calcium channel agonist was greater than that of DR kidneys, and this difference also was further accentuated by dietary NaCl loading.7 ANF, like calcium antagonists, can also increase the GFR1–3,6 9 and ameliorate the effects of vasoconstrictors.10 Unlike calcium antagonists, however, ANF manifests a partial agonist property within the renal circulation,1,8 possibly on the efferent arteriole. In the present work, we examined the possibility that renal responses to ANF, like calcium channel agonists and antagonists,5–7 may be modulated by genetic susceptibility to NaCl-induced hypertension and that the presence of hypertension may further modulate renal responsiveness. We also examined the amelioration or reversal by ANF of the adverse effect of norepinephrine on glomerular filtration — another similarity to calcium antagonists.8

Materials and Methods

We obtained male DS and DR from the Brookhaven National Laboratory (Upton, NY, USA). Initially, the rats were maintained on chow containing 0.3% NaCl. Subgroups of DS and DR then were switched to either a low salt regimen consisting of 0.13% NaCl chow (Teklad, Madison, WI, USA) or a high salt regimen consisting of 1% NaCl in the drinking water and the same 0.13% NaCl chow. They were maintained on either of these regimens for 4 weeks prior to experimentation. At the time of use, the DS were on average...
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 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 perfusate 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 values. After allowing 5 minutes for the restabilization of RVR, specimens were obtained for two final 5-minute periods.

Urine volumes were estimated gravimetrically. Inulin was analyzed by a resorcinol method, and sodium was measured by cesium-standardized flame photometry. The inulin clearance provided an estimate of the GFR. RVR was computed as the quotient of the renal perfusion pressure divided by perfusate flow. The two values for each phase agreed within 15% and were averaged. Statistics were done with these averages.

Statistical comparisons between experimental and control measurements or between different experimental phases within the same group were done by paired t test. Intergroup comparisons were done when a preliminary analysis of variance indicated that significant intergroup differences existed (p < 0.05). The Newman-Keuls multiple comparison test then was used for all possible comparisons at a significance level below 0.05; the Bonferroni test was used for fewer comparisons. Data also were analyzed by a multivariate analysis of variance procedure with repeated measures. This analysis simultaneously contrasted the effects of Dahl status (DS or DR) and salt intake (low or high). Profile analysis tested the effects of Dahl status and salt intake on sequential and nonsequential changes in GFR, RVR, and sodium excretion between the different phases. Results are expressed as means ± SEM.

Results
During Inactin anesthesia, mean arterial pressure (MAP) of the DS fed high NaCl (DS/Hi) averaged 143 ± 4 mm Hg, a value significantly greater than that of the DS fed low NaCl (DS/Lo; 125 ± 3 mm Hg) and of the DR/Hi and Dr/Lo (122 ± 5 and 120 ± 4 mm Hg, respectively; p = 0.012). Baseline RVR values of Dahl rat kidneys were significantly greater in DS than in DR (p = 0.016) and also significantly greater in the low NaCl than in the high NaCl groups (p < 0.001). AP II treatment decreased RVR in both of the high NaCl groups (p < 0.05) but in neither of the low NaCl groups; RVR of DR/Lo kidneys increased slightly but significantly after AP II treatment (Figure 1). Sufficient norepinephrine 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 (p = 0.229).

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-

![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.](image-url)
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.

significantly greater 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 ± 60 µl/min for DS/Hi and 248 ± 63 µl/min for DR/Hi kidneys. The norepinephrine-induced decrement in GFR averaged 202 ± 36 µl/min for DS/Lo kidneys and 275 ± 54 µl/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 (FE₅) 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 ± 1.7 µEq/min in DR/Hi kidneys but only by 1.3 ± 0.4 µEq/min in DS/Hi kidneys (p = 0.016). AP II increased absolute sodium excretion by 2.9 ± 0.5 ± 0.4 µEq/min in DR/Lo kidneys and by 0.34 ± 0.11 µEq/min in DS/Lo kidneys (p = 0.019). Factoring by the GFR, FE₅ of DR/Hi kidneys increased from a control value of 3.0 ± 0.7% to 6.2 ± 1.0% after AP II treatment. FE₅ of DS/Hi kidneys increased from a control value of 0.87 ± 0.10% to only 1.56 ± 0.29% after AP II treatment. These changes in FE₅ differed significantly between the DS and DR kidneys (p = 0.004). FE₅ of DR/Lo kidneys increased from a control value of 4.98 ± 0.82% to 9.11 ± 1.2% after AP II treatment, whereas FE₅ of DS/Lo kidneys increased from a control value of 1.39 ± 0.25% to 2.07 ± 0.49% after AP II treatment. The increase in FE₅ 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 FE₅ of either of the high NaCl Dahl kidney groups, but it decreased DR/Lo kidney FE₅ 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 pressure5,12 and ultrafiltration coefficient.13 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.5,14,15 Our results indicate that the action of ANF on the GFR is increased by NaCl load-
increased RVR and GFR responses to a dihydropyri-
remains that operation of the intrarenal renin-angioten-
system may favor the generation of angiotensin II
lished observations, 1987). Therefore, the possibility
kidneys (T.H. Steele and L. Challoner-Hue, unpub-
response of low NaCl kidneys to AP II, but instead
tor antagonists into the perfusate does not increase the
converting enzyme inhibitors and angiotensin II recep-
pressure with added albumin results in a brisk increase
merization of ANF action on the GFR can occur inde-
absence of angiotensinogen from the perfusate. How-
giotensin II may have antagonized the actions of AP II
loading.20
high AP II concentration and a different ANF molecu-
lar species may explain the differences between our
rants,3 AP II was ineffective at eliciting a substantial
natriuresis by the isolated DS kidney, even in the pres-
the DS.28
Because the intrarenal renin-angiotensin system is
activated during salt restriction,22 renally generated an-
giotensin system within the isolated kidney is un-
characterized. 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.21 In addition, the incorporation of angiotensin
converting enzyme inhibitors and angiotensin II recep-
tor 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-angioten-
sin 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-
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

Hypertension. 1988;11:745-749
doi: 10.1161/01.HYP.11.6.745

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/11/6_Pt_2/745

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/