Transplantation of Enalapril-Treated Kidneys Confers Persistent Lowering of Arterial Pressure in SHR
Corry Smallegange, Robert L. Kline, Michael A. Adams

Abstract—The kidney plays a critical role in regulating the level of arterial pressure and in the pathogenesis of hypertension. Important evidence has come from studies in which hypertension is generated by transplanting kidneys from genetically hypertensive rats into normotensive recipients, suggesting that the level of blood pressure is strongly influenced by the genetic background of the kidney. We hypothesized that pharmacotherapy could modify specific properties intrinsic to the kidney such that after transplantation, there would be persistent changes in the level of arterial pressure. We determined that angiotensin-converting enzyme inhibitor treatment (enalapril) in spontaneously hypertensive rats induced both a persistent 17% reduction of mean arterial pressure and a persistent change in the kidney. This persistent change in the circulation could be completely transferred to untreated spontaneously hypertensive rats by kidney transplantation; ie, mean arterial pressure in untreated spontaneously hypertensive rat recipients was persistently lowered after transplantation of a kidney from a previously treated spontaneously hypertensive rat donor. In addition, the persistent lowering of mean arterial pressure after enalapril treatment could be completely abolished by implanting an untreated kidney, thereby revealing the importance of the kidney-specific changes. Furthermore, after within-group transplantations, there were no changes in the level of arterial pressure; ie, a 16% difference in mean arterial pressure remained between the 2 groups. The findings revealed that drug-induced changes specific to the kidney determined the level of arterial pressure, thereby suggesting the kidney should be a key therapeutic target for pharmacotherapy. (Hypertension. 2003;42:932-936.)

Key Words: transplantation, renal ± renin-angiotensin system ± rats, spontaneously hypertensive ± arterial pressure ± angiotensin-converting enzyme inhibitors

According to a model of the circulation proposed by Guyton,1 regulation of arterial pressure occurs at a level that permits fluid balance to be achieved. Specifically, this conceptual framework emphasizes that the kidneys are critical in regulating sodium and water balance and the long-term level of arterial pressure. In part, the operating range of arterial pressure is determined by the pressure-natriuresis mechanism, a process in which there is a direct relation between arterial pressure and urinary sodium excretion. Resetting of this relation to higher levels has been proposed to be a principle cause of hypertension, particularly in spontaneously hypertensive rats (SHR).1,2 Studies by Folkow and others3–6 have suggested that differences in vascular structure underlie some of the circulatory alterations in SHR, such as the shift in the operating range of the pressure-natriuresis mechanism.7

Regardless of the mechanism, the kidneys of SHR have been shown to have a transplantable, genetically determined abnormality that confers the donor’s increased level of arterial pressure onto the circulation of the normotensive recipient, usually a hybrid strain.8–10 A similar effect of transplantation has also been described in humans, wherein arterial pressure after kidney transplantation is shown to be markedly influenced by the cardiovascular status of the donor.11 Most studies to date have emphasized that genetic background is one of the most important factors in this response to transplantation.11 Rettig et al11 reported that even when kidneys were transplanted from SHR that had received antihypertensive treatment (ie, such that blood pressures were not allowed to rise to hypertensive levels), the arterial pressure of the recipients rose to hypertensive levels soon after the kidney was transplanted.11 The authors interpreted these results as indicating that although the treatment had controlled arterial pressure and protected the kidney from hypertension-induced damage, reestablishment of hypertension in the recipient was evidence that the genetic background of the donated kidney was a critical factor.

Previous studies have shown that angiotensin-converting enzyme (ACE) inhibitors are particularly efficacious antihypertensive agents in SHR regarding their capacity to induce both structural and functional changes throughout the circulation.13 Importantly, not only do these agents produce a
robust lowering of arterial pressure during treatment but also these effects persist long after cessation of treatment. In the present study, our main objective was to determine whether these drug-induced, persistent changes in the circulation specifically involved the kidney. To accomplish this, we performed studies in which we determined: (1) the impact of prolonged enalapril treatment on SHR on the level of mean arterial pressure (MAP) after treatment was stopped, both before and after uninephrectomy, and after within-group kidney transplantations; (2) the impact on the level of MAP when kidneys were cross-transplanted between previously treated and untreated SHR; and (3) the associated changes in the MAP-salt balance relation as an indicator of the responsiveness of the kidney in treated, untreated, and postransplantation animals.

Methods

Animals and Treatments
Three- to 4-week-old, male SHR (n=56; Charles River, Montreal, Canada) in controlled housing (21°C; 12:12-h light/dark cycle) were given enalapril (n=28; 25 mg · kg⁻¹ · d⁻¹ in drinking water) or tap water (controls, n=28) for 10 weeks. After surgery, all rats were given antibiotics (Tribrissen injectable, 24% at 0.5 mL/kg SC daily for 7 days) and buprenorphine (Buprenex injectable at 0.3 mg q12h SC), as required. All procedures were in accordance with the Canadian Council on Animal Care.

Assessment of MAP
Under isoflurane anesthesia, SHR were implanted with a radiotelemetric pressure transducer (model TA11PA-C40, Data Sciences Inc). MAP was determined from data collected every 5 minutes (15 seconds, 150 Hz) by a digital radio signal received by units under each cage (model RA1010, RA1020, or RPC-1; Data Sciences) and transferred by a consolidation matrix (BCM100, Data Sciences) to the data acquisition system (Dataquest LabPRO or Dataquest ART, Data Sciences).

Assessment of MAP After Uninephrectomy
ACE inhibitor–treated and control animals were implanted with pressure transducers at 11 weeks of age. At 13 weeks of age, ACE inhibitor therapy was stopped. Two weeks later (15 weeks of age), the animals were uninephrectomized (UNX) under isoflurane anesthesia through a 1-cm incision over the left kidney. The left renal artery and vein and the ureter were isolated and ligated, and the left kidney was removed.

Kidney Transplantation
The kidney transplantation procedure was modified from that of Zhang et al and involved isoflurane anesthesia. In donor rats, the left kidney was isolated and perfused with ice-cold, lactated Ringer’s solution. Recipient native right kidneys were removed, and the inferior vena cava and aorta were cross-clamped (time <40 minutes) before end-to-side anastomosis (10-0 monofilament continuous sutures) of the renal artery and vein to the aorta and vena cava, respectively. Ice-cold, lactated Ringer’s solution irrigated the donor kidney during the anastomosis, and microfibrillar collagen (Avitene) was placed around the anastomosis site. The ureter was sutured end-to-end (10-0 monofilament). Seven days later, the left native kidney was removed under anesthesia, and a telemetric pressure transducer was implanted distal to the transplanted kidney (ie, each SHR had 1 kidney from this point on). The groups studied were as follows:

- treated²-control: previously treated SHR that received a kidney from a control SHR donor;
- treated²-untreated: untreated SHR that received a kidney from a previously treated SHR donor;
- control²-untreated: untreated SHR that received a kidney from an untreated SHR donor; and
- treated²-pretreated: previously treated SHR that received a kidney from a previously treated donor.

Figure 1. Ten-week ACE inhibitor treatment (25 mg · kg⁻¹ · d⁻¹ PO) induced a persistent lowering of MAP after treatment withdrawal. During the last 2 weeks of treatment, blood pressure had decreased significantly (P<0.001) in the treated group (97±1.1 mm Hg, n=5) compared with untreated littermates (145±1.6 mm Hg, n=5). After withdrawal of treatment, blood pressure rose slightly (116±1.0 mm Hg) but remained significantly lower than in untreated littermates (147±1.0 mm Hg). This degree of persistent lowering remained unchanged, even after UNX. At the end of the study, MAP had still not returned toward levels found in the untreated group (156±1.2 mm Hg in untreated rats vs 123±0.7 mm Hg in treated rats).

MAP-Sodium Balance Relation In Vivo
Changes in MAP after transplantation were characterized after sodium restriction, sodium challenge, and short-term treatment with an angiotensin type 1 antagonist (losartan, 30 mg · kg⁻¹ · d⁻¹×3 days). Salt manipulations involved 3 days of a normal-salt diet (0.4% Na⁺), 3 days of a low-salt diet (Ren’s rat chow, 0.03% Na⁺), and 5 days of a high-salt diet (normal diet+1% NaCl solution). For all diets, food and fluid were available to the animals ad libitum.

Statistical Analysis
Significant differences between groups were assessed by ANOVA, followed by a comparison of means with a Newman-Keuls post hoc test (P<0.05 was used). Data are presented as either mean±SD or mean±SE, as indicated.

Results

Characterization of MAP
Both at the end of characterization (−33%, P<0.001) and during the off-treatment period (−21%, P<0.001), enalapril significantly decreased MAP compared with untreated controls (Figure 1). UNX performed during the postenalapril treatment period (2 to 4 weeks) did not significantly alter either the absolute level of MAP in control or treated SHR or the difference in MAP (−22%, P<0.001) between control and treated animals (Figure 1). These hemodynamic data confirmed that there was no significant effect on MAP after the...
Assessment of Kidneys After Transplant Procedure

Surgical failure can be a potential problem in microsurgical renal transplantation, with renal artery stenosis and kidney failure followed by secondary hypertension being the most significant complications.\textsuperscript{11} There was no evidence of these problems in the present study. Blood pressure profiles in the within-treatment transplant study clearly demonstrated that the transplant procedure itself did not induce changes in the level of arterial pressure. In addition, the plasma levels of both creatinine (donors=53±8.0 μmol/L, n=9; recipients=50±4.7 μmol/L, n=10) and urea (donors=9.2±1.34 mmol/L, n=9; recipients=8.6±1.3 mmol/L, n=10) were found to be similar in all rats regardless of whether they were donors or recipients. These data help confirm the viability of the transplantation procedures.

Assessment of the MAP-Sodium Balance Relation In Vivo

As a further assessment of circulatory status after transplantation, the MAP responses to manipulations of dietary salt intake (low, normal, and high) and to antagonism of the angiotensin type 1 receptor (losartan) were also assessed. The depressor response to short-term (3-day) losartan treatment in control rats that received treated kidneys (control\textsubscript{D→control}, 14.4±5.8%) and in treated rats that received control kidneys (treated\textsubscript{D→control}, 18.3±5.9%) was not significantly different. Importantly, a similar depressor response to short-term losartan treatment was found in control rats that received control kidneys (control\textsubscript{D→control}, 16.1±1.61%) and in treated rats that received treated kidneys (treated\textsubscript{D→treated}, 17.5±1.47%).

In response to manipulations of dietary salt intake, the MAP-urinary sodium relation (Figure 4) was significantly shifted leftward (≈11% to 17%) in untreated rats that received a kidney from previously treated rats (control\textsubscript{D→treated}) compared with previously treated rats that received a kidney from untreated SHR (treated\textsubscript{D→control}). Furthermore, there was a similar shift in the MAP-urinary sodium relation in the within-group transplantation study. That is, the difference between treated rats that received treated kidneys (treated\textsubscript{D→treated}) and control rats that received control kidneys (control\textsubscript{D→control}) ≈11% to 16%) was similar to that found in the between-treatment transplantation study.

Discussion

A major finding of this study is that antihypertensive treatment with an inhibitor of the renin-angiotensin system in-
duced changes in the kidney directly linked to persistent, long-term lowering of arterial pressure. Specifically, this relation was revealed by complete cross-over of the arterial pressure profiles after cross-transplantation of kidneys between the untreated, hypertensive SHR and previously treated SHR. In fact, the difference in arterial pressures in the cross-transplanted groups (−17% for between groups) was the same as the difference between intact and UNX, untreated and treated animals (−18% after UNX, −16% for within the group). These findings reveal that the antihypertensive drug-induced, persistent decrease in arterial pressure is related to changes that appear to be kidney specific, because in either case of cross-transplantation, the origin of the kidney dictated the subsequent effect on arterial pressure.

It is an important finding in all 3 studies that the magnitude of the persistent decrease in arterial pressure in SHR with enalapril-treated kidneys versus the untreated group was similar. In fact, even the absolute levels of MAP of the 2 groups were comparable (115 to 126 mm Hg for all SHR with treated kidneys; 140 to 150 mm Hg for all untreated kidneys). Specifically, the consistency shown for both the relative and absolute arterial pressure measurements provides strong evidence for the importance of the kidney-specific, drug-induced changes or the lack thereof in the persistent effect on MAP. Thus, the increase in MAP toward untreated, hypertensive levels in previously treated SHR that received an untreated kidney also indicates that the effect of the kidney on the particular level of arterial pressure was not altered by the other known drug-induced changes in the circulation (eg, regression of cardiac and regional vascular structure, normalization of baroreflex mechanisms, and improvement in endothelial dysfunction). Previously, the development of hypertension in hybrid normotensive rats transplanted with SHR kidneys was interpreted as primarily being a consequence of the hypertensive genetic background of the donor kidney. The present findings demonstrate that, despite the genetic background, the kidney-specific properties that confer hypertension are modifiable by drug treatment. That is, enalapril treatment has effectively modified the age-related changes in the SHR kidney, such that after transplantation, hypertension in the untreated recipient is reversed. Whether enalapril treatment is also able to produce persistent, transplantable changes in the adult kidney will require further experimentation.

In previous kidney transplantation studies, immunologic incompatibility (eg, graft rejection) between hypertensive and normotensive animals or between hypertensive and hybrid strains was thought to be a significant factor in the development of hypertension after the procedure. In the present study, this issue was largely circumvented by having all transplantations occur between littermates. Tissue compatibility was further confirmed in that there was no noticeable impact on the level of MAP in the within-group transplantation study. In addition, the stability of MAP after UNX in both control and treated SHR showed that compensatory renal growth does not influence the level of MAP. This assessment was important because compensatory renal growth can occur as early as 48 hours after UNX.

Numerous investigations have demonstrated that hypertension can be transferred by transplanting kidneys from genetically hypertensive donors to normotensive recipients. Although many of these previous investigations suggested that extrarenal mechanisms are involved in promoting hypertension in SHR, the data from the present studies emphasize the importance of properties intrinsic to the kidney. In particular, the current findings reveal that the renal mechanisms that determine the level of arterial pressure are modifiable and persist after pharmacotherapeutic intervention. Furthermore, although hypertension in SHR is strongly linked to genetic background, the present investigation demonstrates that the kidney-specific effects of enalapril treatment prevail. Further studies need to be performed to determine whether the enalapril effects on the transplanted kidney and thereby on the arterial pressure of the recipient animal are linked to changes in renal vascular structure.

Perspectives

These studies provide a rational basis for developing therapeutic strategies that selectively target the kidney. The findings further suggest that pharmacologic modification of specific properties of the kidney is a possible therapeutic benefit, even in hypertension with a strong genetic basis. Finally, although further investigation with other therapeutic agents is needed, the results suggest that the persistent lowering of arterial pressure that occurs after antihypertensive treatment is an effect that might be accounted for by changes specific to the kidney.

Acknowledgments

Funding for this study was provided by a grant from the Canadian Institutes of Health Research and from the Heart and Stroke Foundation of Ontario. Enalapril was graciously provided by Merck-Frosst, Canada.

References


Transplantation of Enalapril-Treated Kidneys Confers Persistent Lowering of Arterial Pressure in SHR
Corry Smallegange, Robert L. Kline and Michael A. Adams

Hypertension. 2003;42:932-936; originally published online September 29, 2003; doi: 10.1161/01.HYP.0000092883.16529.84
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 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/42/5/932

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/