Persistent Lowering of Pressure by Transplanting Kidneys From Adult Spontaneously Hypertensive Rats Treated With Brief Antihypertensive Therapy

Corry Smallegange, Taben M. Hale, Terri L. Bushfield, Michael A. Adams

Abstract—Kidney function is critical in determining the level of arterial pressure and in the pathogenesis of hypertension. Important evidence comes from studies in which the level of blood pressure is dictated by the donor when kidneys are transplanted between genetically hypertensive and normotensive rats. We have hypothesized that pharmacotherapy modifies specific properties of the kidney, particularly the vasculature, such that after kidney transplantation, there are persistent changes in the level of arterial pressure. Consistent with previous studies, a 2-week aggressive treatment of adult (15 weeks) spontaneously hypertensive rats with an angiotensin-converting enzyme inhibitor (enalapril) combined with a low-salt diet induced a persistent change in the kidney and a decrease in arterial pressure (18%). These persistent changes in arterial pressure could be completely transferred to untreated adult spontaneously hypertensive rats by kidney transplantation (ie, pressure in untreated rats was decreased after transplantation of a kidney donated from a previously treated rat). Further, the importance of kidney-specific changes was demonstrated by finding that the treatment-induced lowering of arterial pressure was completely reversed by transferring an untreated kidney into a previously treated rat. The specific treatment-induced changes to the kidney included a decrease in structurally based renal vascular resistance that was similar to the persistent lowering of arterial pressure. These data provide evidence for a link between the treatment-induced changes in kidney vascular structure and the persistent lowering of arterial pressure. The findings also suggest that a key pharmacotherapeutic target in hypertension should be kidney-specific changes, such as renal vascular structure. (Hypertension. 2004;44:89-94.)

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

It is well established that long-term treatment of either young or adult spontaneously hypertensive rats (SHRs) with angiotensin-converting enzyme (ACE) inhibitors induces a lowering of mean arterial pressure (MAP) and a downregulation of cardiac and vascular structure that persists even after treatment is stopped.1-5 In addition, a causal relationship between altered vascular structure and persistent change in MAP has been suggested on the basis of findings that the reduction of structurally based vascular resistance properties after treatment with renin–angiotensin system (RAS) inhibitors is of similar magnitude to the persistent lowering of arterial pressure.1,4,6-8 Given the important role of the kidneys in determining the “set point” of arterial pressure, it may be that it is regression of renal vascular structure that is key to inducing this reversal of hypertension. For example, Dukacz et al showed that ACE inhibitor-induced persistent lowering of arterial pressure in SHRs was associated with persistent alterations in intrarenal hemodynamics that result in a persistent shift in the set point of the pressure–natriuresis response toward lower arterial pressures.9 Although not direct evidence, these data suggested that downward remodeling of the renal vasculature may be the critical kidney-specific change responsible for persistent changes in pressure after RAS inhibitor treatments.3 In fact, we demonstrated recently that MAP can be persistently lowered in untreated SHRs by transplanting a kidney from an SHR that had previously received long-term ACE inhibitor treatment during the rapid growth phase of development.3

It was suggested previously that long-term antihypertensive treatment in young growing animals was the most effective means of inducing persistent changes in the circulation.4,10-12 However, more recent studies have demonstrated that persistent lowering of MAP can occur with as little as 2 weeks of treatment with inhibitors of the RAS in adult SHRs.1,13 The objective of the present study was to determine whether this brief, aggressive ACE inhibitor treatment (only 2 weeks of therapy) in adult SHRs with fully established hypertension can also induce kidney-specific changes that are transplantable (ie, can transplanting a kidney into an untreated SHR after only a 2-week treatment dictate a lower

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arterial pressure in the recipient?). In addition, to further investigate the mechanism of the kidney-specific changes, we characterized structurally based vascular resistance properties in the kidney after cessation of the 2-week treatment.

Methods

Animals and Treatments

Ten-week-old male SHRs (n = 38) were obtained from Charles River Laboratories (Montreal, Canada) and housed individually (21 ± 1°C, 12 hour light/dark cycle). Access to food and water was ad libitum. From 15 to 17 weeks of age, 1 group was treated with the ACE inhibitor enalapril (30 mg·kg⁻¹·day⁻¹; Sigma) in the drinking water combined with a low-salt diet (ELS; Purina rodent chow 0.04% Na⁺). From day 6 to 14 of treatment, animals were given access to regular rodent chow (0.4% Na⁺ Purina) for only 4 hours (this provided ~7 g of chow containing 30 mg sodium per day and stabilized MAP from day 6 onward, as described previously). Control (CON) animals received tap water and regular chow.1 The animals were used as follows: (1) the long-term nontransplant, radiotelemetry involved 16 SHRs (12 CON group, 4 ELS group; Figure 1); (2) 16 SHRs were involved in the crossover transplantation as donors (n = 8; euthanized) and recipients (n = 8) (Figures 2 and 3); and (3) 6 SHRs were used in the analysis of structurally based renal vascular resistance properties (Figure 4).

Experimental procedures were approved by the Queen’s University Animal Care Committee in accordance with guidelines established by the Canadian Council on Animal Care.

Kidney Transplantation Experiments

Crossover kidney transplantation was performed between groups of treated and untreated animals 2 weeks after stopping ELS treatment. Animals treated previously with ELS received a CON kidney (ELSkn-CONn = 4), whereas CON animals received a previously treated kidney (CONkn-ELSn = 4). Previous crossover kidney transplantation studies from our laboratory and from others have been done using either littermates or hybrid animals to prevent immunological tissue rejection after transplantation. In the present study, we characterized the response of crossover kidney transplantation using nonlittermate, within-strain, SHR-to-SHR transfers and found there to be no immunological problems after within-strain kidney transplantation.

Transplantation Procedure and MAP Assessment

The kidney transplantation procedure, as described previously, was based on a modification of the procedure of Zhang et al. In brief, all surgery used isoflurane anesthesia (2 L/min; Janssen). The donor’s left kidney was perfused with ice-cold lactated Ringer’s solution and removed. The recipient’s native right kidney was removed, and an end-to-side anastomosis of the renal artery, vein, and ureter was performed with the donor kidney. Finally, a radiotelemetric transducer (model TA11PA-C40; Data Sciences) was implanted into the abdominal aorta as described previously. Seven days after transplantation, the remaining left native kidney was excised. For both procedures, buprenorphine (Temgesic, 3 mg/kg; Reckitt and Coleman Pharmaceuticals) was administered for postoperative pain, as required. Antibiotics (injectible tribrissen, 24% at 8 mL/kg SC) were given for 7 days after surgery. Animals were given 1 week to recover from the uninephrectomy surgery before MAP was recorded. From that point, MAP was recorded continuously for 18 to 19 weeks via radiotelemetry (Dataquest IV, Version 2; Data Sciences) as described previously.

In Vivo Assessment of the Relationship Between MAP and Sodium Balance

We performed studies involving sodium restriction, sodium challenge, and an angiotensin II type-1 receptor antagonist losartan treatment to compare the effects of kidney cross-transplantation between CONkn-ELS and ELSkn-CON groups. This was performed 12 to 13 weeks after kidney transplantation (aged 32 to 33 weeks) and involved 3 days of normal-salt diet (NS = Purina rodent chow, 0.4% Na⁺ and tap water), 3 days of low-salt diet (LS = Purina rodent chow, 0.04% Na⁺ and tap water), and 5 days of a high-salt diet (HS = Purina rodent chow, 0.4% Na⁺ and water containing 1% NaCl). One week after the end of the HS diet, animals were treated for 5 days with losartan (30 mg·kg⁻¹·day⁻¹ PO; donated by Merck Frosst Labs Canada, Inc., Point-Clare, Quebec). Throughout all diet regimens, access to food and fluid was ad libitum.

Kidney Status After Transplantation

To ensure there was no immunological incompatibility, we assessed the health and recovery of the kidneys as described previously. In brief, just before euthanasia, we examined the kidneys macroscopically and after transverse sectioning, and took an aortic blood sample (5 to 7 mL, 5000 rpm, 15 minutes) to determine plasma creatinine and urea levels (Department of Clinical Chemistry, Kingston General Hospital) of the donor animals at the time of transplantation (aged 19 weeks) and the recipient animals at the end of the study (aged 36 to 37 weeks), as described previously.

Assessment of Cardiac Structure

Assessments of cardiac mass were conducted in the donor animals at the time of transplantation (19 weeks) and in the recipient animals at the end of the study (36 to 37 weeks). Changes in cardiac structure were determined from the ratio of the left ventricle plus septum to body weight (LVS:BW) and the right ventricle to body weight ratio (RV:BW).

Structurally Based Vascular Resistance Properties: Kidney

In separate groups of SHRs (n = 6), using an in situ preparation, we assessed renal structurally based vascular resistance 2 to 3 weeks after stopping the ELS therapy and in age-matched untreated rats. Changes in renal interstitial hydrostatic pressure (RIHP) directly correlate with alterations in renal perfusion pressure (RPP) because the renal medullary circulation is not autoregulated. The assessment of the renal vasculature is based on findings by Folkow et al. but also includes direct determination of RIPP according to Roman et al. In brief, under thiobutabarbital anesthetic (Inactin, 100 mg/kg IP; RBI), the abdominal aorta was exposed via a midline incision. Ligatures were used to eliminate all nonrenal flow (ie, only to the left kidney) and the left ureter was cannulated (pulled PE-50 tubing). Heparin (1000 IU/kg IV; Sigma) was administered before the aorta was cannulated retrogradely. The catheter tip was advanced to the distal edge of the aortic origin of left renal artery and tied. An 18-g catheter was inserted and tied into the aorta proximal to the left renal artery for recording RPP. In a temperature-controlled chamber (37.5°C), the left renal vein was severed to allow for outflow and the spinal cord severed to remove neural influences. Immediately afterward, the kidney was perfused (1.0 mL/min) with oxygenated dextran–Tyrode containing the vasodilator sodium nitroprusside (100 μmol/L). An electrocautery needle (26 gauge) was used to create a 3-mm hole in the longitudinal axis of the kidney for insertion (glued with cyanoacrylate) of a catheter for RIPP measurement (PE-50 with 2- to 3-mm polyethylene matrix, pore size 70 μm; Bel-Art Products). The patency of the RIPP catheter was validated by the appropriate RIPP response after infusion of a bolus (~100 μL) of saline (ie, a sharp 10 to 20 mm Hg increase in RIPP followed by return to baseline in ~30 seconds). The RPP–RIPP relationship was determined for various flow rates (0.5 to 12 mL/min per 100 g of body weight) and plotted at an RIPP of 4, 5, and 10 mm Hg by linear interpolation from the collected data.

Results

Characterization of Changes in MAP

MAP was similar in both groups before the initiation of treatment (ELS 141 ± 6.4 mm Hg versus CON 142 ± 6.2 mm Hg) (Figure 1). During the first 6 days of ELS
treatment, MAP fell rapidly in the ELS group, decreasing 52% from the pretreatment values (68 ± 2.4 mm Hg) and stabilized at this level until the end of the period of drug administration. After cessation of treatment, blood pressure rose over several days but remained at a level that was ∼18% lower than in untreated SHRs. To assess the overall impact of this treatment, MAP was assessed 19 weeks after stopping treatment (36 to 37 weeks of age). At this chronic post-treatment time point, the MAP of the previously treated SHRs was still ∼18% below levels in untreated animals (CON 157 ± 14.0 mm Hg versus ELS 129 ± 4.6 mm Hg). In fact, more than 20 weeks after starting the treatment, the MAP of the previously treated SHRs was even lower than (∼8%) their initial pretreatment level.

Transplantation of kidneys between CON- and ELS-treated SHRs resulted in a full crossover of MAP profiles (Figure 2). After transplantation of CON kidneys into the previously treated SHRs, MAP was significantly increased (ELSK 155 ± 2.2 mm Hg) relative to the CON SHRs that received a kidney from a previously treated rat (CONK 127 ± 2.5 mm Hg). The overall impact of transplanting a previously treated kidney into a CON animal was a lowering of MAP (17.4 ± 2.83%), a difference that was still evident even 5 months after surgery (Figure 2).

Overall, the impact of a uninephrectomy procedure was minimal. Specifically, after the uninephrectomy/transducer implantation surgery at 19 to 20 weeks of age (2 weeks after treatment), blood pressure rose transiently and minimally, returning to levels observed before uninephrectomy. Thus, uninephrectomy did not have any lasting effects on arterial pressure in either previously treated or CON SHRs.

In Vivo Assessment of the MAP–Sodium Balance Off Treatment

The magnitude of the change in MAP with variations in dietary sodium intake during the off-treatment period was not significantly different between transplant animals that received a treated or an untreated kidney during LS and HS dietary manipulations. B, The MAP–sodium balance relationship was shifted in parallel to lower levels of MAP by ∼31 mm Hg in the CONK-ELS group compared with the ELSK-CON group. **Significant difference from ELSK-CON (P < 0.05).

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In Vivo Assessment of the MAP–Sodium Balance Off Treatment

The magnitude of the change in MAP with variations in dietary sodium intake during the off-treatment period was not significantly different between transplant animals that received a treated or an untreated kidney during LS diet (ELSK-CON −7.3% ± 1.72%, CONK-ELS −4.4% ± 1.93%) or HS diet (ELSK-CON 0.98% ± 2.72%, CONK-ELS 0.20% ± 3.70%) (Figure 3). Losartan challenge (not shown in figure) also resulted in a similar depressor response (ELSK-CON −67% ± 3.79%, CONK-ELS −59% ± 5.36%) between groups. However, the one important difference was that the set point of the MAP–sodium balance relationship was parallel shifted to a level of arterial pressure in transplant animals that received a treated kidney during LS diet (Figure 3).

Figure 1. MAP ± SD in CON (n = 12) animals and animals treated with enalapril (30 mg · kg⁻¹ · day⁻¹) for 2 weeks in combination with an LS diet (n = 4). ELS induced a persistent lowering of pressure after treatment was stopped. The shaded bar indicates the treatment period. **Significant difference compared with CON over the off-treatment period (day 22 to 152; P < 0.001).

Figure 2. MAP ± SD “follows the kidney” after cross-transplantation of kidneys between animals with ACE inhibitor-induced persistent lowering of pressure and CON animals. The shaded bar indicates the treatment period. Day 42 is at 21 weeks of age and is also the time point at which transplantation and uninephrectomy occurs. **Overall significant difference of the ELS group compared with CON (P < 0.05). †Significant difference between ELSK-CON and CONK-ELS (P < 0.05).

Figure 3. A, MAP ± SD profiles of ELSK-CON and CONK-ELS during LS and HS dietary manipulations. B, The MAP–sodium balance relationship was shifted in parallel to lower levels of MAP by ∼31 mm Hg in the CONK-ELS group compared with the ELSK-CON group. **Significant difference from ELSK-CON (P < 0.05).
pressure 31 mm Hg lower in the CON\textsubscript{K\textgreater{}ELS} group compared with the ELS\textsubscript{K\textless{}CON} group (Figure 3B).

**Determination of Global Renal Function**

Plasma creatinine and urea levels for the transplant recipient animals (creatinine 40.0±10.53 μmol/L, urea 9.0±1.99 mmol/L) were not significantly different from those found in the donor animals that did not undergo the transplantation procedure (creatinine 46.0±22.30 μmol/L, urea 8.2±1.18 mmol/L). In addition, kidneys were sectioned laterally for gross examination. There were no anatomical differences evident between the treatment groups.

**Changes in Cardiac Structure**

The assessments of cardiac structure conducted in the donor animals at the time of the transplantation procedure revealed that the LVS:BW ratio of the donor animals treated previously with ELS (2.2±0.13 g/kg) was significantly reduced compared with the untreated donor animals (2.5±0.13 g/kg). Similarly, at the end of the study, the LVS:BW ratio in the CON animals that had received a treated kidney (2.2±0.05 g/kg) was significantly reduced compared with the treated animals that had received a CON kidney (2.4±0.09 g/kg).

There were no significant differences between the RV:BW ratios of CON (0.54±0.065 g/kg) and previously treated donor animals (0.55±0.358 g/kg) or between the RV:BW ratios in CON\textsubscript{K\textgreater{}ELS} (0.42±0.010 g/kg) and ELS\textsubscript{K\textless{}CON} (0.46±0.111 g/kg) groups.

**Structurally Based Vascular Resistance Properties in the Kidney**

Overall, the ELS treatment induced a persistent decrease in structurally based renal vascular resistance properties, as demonstrated by the significant leftward shift of the RPP–RIHP relationship (\(P<0.01\); Figure 4). Specifically, 2 to 3 weeks into the off-treatment period, compared with CON animals, the operating range of RPP was shifted to lower perfusion pressures in previously treated animals at RIHP levels of 4 mm Hg (29.0%±8.74%), 5 mm Hg (30.5%±8.89%), and 10 mm Hg (35.2%±11.42%). Data from untreated SHRs were obtained from a parallel series of experiments in CON animals (\(n=11\)).

**Discussion**

The major finding of this study is that only 2 weeks of an aggressive antihypertensive therapy in adult SHRs produced persistent lowering of arterial pressure, an effect that was transferred into an untreated SHR by transplanting a kidney from a previously treated donor into an untreated rat. In addition, the converse was also true, in that arterial pressure completely reverted back to hypertensive levels after replacing the kidneys in the treated animals with a single kidney from an untreated SHR. Furthermore, the 2-week antihypertensive treatment produced a significant decrease in structurally based vascular resistance properties of the kidney that was of the same magnitude as the persistent lowering of MAP. Given that the changes in MAP and renal vascular resistance are similar, the data suggest a link between the treatment-induced kidney vascular effects and the persistent lowering of arterial pressure.

In confirming our previous transplantation results,\textsuperscript{3} the present findings reinforce the understanding that the mechanisms, which confer a persistent lowering of arterial pressure after antihypertensive treatment, reside within the kidney. That is, by transplanting a kidney from a treated SHR into an untreated animal, the extent of MAP lowering was the same as the effect of the antihypertensive treatment in intact SHRs. The importance of the kidney-specific changes was further emphasized when transplantation of an untreated kidney into a treated SHR reversed arterial pressure back to hypertensive levels, regardless of the previous drug effects on the rest of the circulation. Thus, although the concept that the level of arterial pressure follows the kidney has been further confirmed, more specifically, the data demonstrate that this time in adult SHRs, antihypertensive treatment directly modifies a renal-specific mechanism that determines the level of arterial pressure.

The experimental kidney transplantation protocol was validated in a previous study from our laboratory.\textsuperscript{3} Because effectively, only 1 kidney is transplanted to bilaterally nephrectomized recipients in these experiments, a group of treated and CON animals were unilaterally nephrectomized (no transplantation) to control for the impact of a solitary kidney or the effect of compensatory renal growth (CRG) on the long-term level of arterial pressure.\textsuperscript{3,16} As in our previous study,\textsuperscript{3,5} no differences in left kidney mass were found between the groups after uninephrectomy, confirming that previous ACE inhibitor treatment does not alter the CRG response. Using the radiotelemetric blood pressure assessment, we also confirmed that uninephrectomy, with the associated CRG, has no effect on the level of arterial pressure in CON or previously treated SHRs.\textsuperscript{3} On the basis of these findings, nonspecific or compensatory changes resulting from the uninephrectomy can probably be excluded as an explanation for the impact of kidney transplantation on the long-term level of arterial pressure.

In previous transplantation studies, immunological incompatibility between hypertensive and normotensive, or between hypertensive and hybrid strains, has been a major
concern when examining the impact of transplantation. \(^{14,16}\) As in our previous study, in the present investigation, immunological compatibility was not an issue because we used a within-strain transplantation design. In addition, to ensure the viability of the kidneys, after transplantation, we determined that plasma creatinine and urea concentrations were similar to the levels in donors before transplantation. We have established previously that a within-strain, within-group kidney transplantation does not alter the long-term level of MAP. \(^3\)

In the ELS diet-treated rats, long after therapy was stopped, there remained a significant shift in the RPP–RIHP relationship toward lower RPP set point of \(\approx\)30%. The downward resetting of this particular aspect of renal function suggests that there was a significant treatment-induced regression of the structurally based vascular resistance properties of the kidney. Other investigations have also revealed, although in intact anesthetized SHRs, a similar persistent shift in the RPP–RIHP relationship after long-term treatment with standard doses of ACE inhibitors. \(^9,36\) In this study, the magnitude of the leftward shift in the RPP–RIHP relationship could completely account for the magnitude of persistent lowering of arterial pressure observed after cessation of treatment. Previous studies support the concept that there is regression of vascular structure in that the same brief aggressive treatment has been demonstrated to cause regression of both the hindlimb\(^1\) and penile\(^1\) vascular beds. What is not known is whether the changes in vascular structure outside of the kidney occur independently of the kidney or are under the direction of the kidney. Regardless, the present results suggest that resetting of the properties of the renal vasculature alone may be sufficient to induce the persistent lowering effect on arterial pressure in an SHR.

**Perspectives**

These findings reveal that only 2 weeks of an aggressive treatment with an ACE inhibitor in adult SHRs can produce kidney-specific changes that result in persistent long-term lowering of arterial pressure. Together with previous studies, these studies also suggest that pharmacotherapy-induced renal vascular structure downward remodeling of the renal vascular tone may be the critical kidney-specific mechanism responsible for the long-term, persistent lowering of arterial pressure. It may be that more selective therapeutic targeting of kidney-specific processes, particularly related to vascular structural properties, could provide the basis for an important treatment strategy in reversing the hypertensive phenotype.

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