Altered Vascular Resistance Properties and Acute Pressure-Natriuresis Mechanism in Neonatal and Weaning Spontaneously Hypertensive Rats

Marina Komolova, Peter Friberg, Michael A. Adams

Abstract—Although it has been extensively scrutinized, the factor(s) involved in the initiation and development of hypertension in spontaneously hypertensive rats (SHRs) remains unresolved. The objective of the present study was to determine whether, early in development, the causal mechanism(s) for the development of hypertension in young SHRs involves an integration of 2 processes, specifically an upregulation of structurally based vascular resistance properties and a rightward shift in the hemodynamic component of pressure-natriuresis. Mean arterial pressure was determined in conscious 4-week-old SHRs and Wistar-Kyoto rats via previously implanted aortic catheters. Structurally based hindlimb vascular resistance properties were assessed in 2- and 4-week-old SHRs and Wistar-Kyoto rats. Renal interstitial hydrostatic pressure was measured after short-term manipulations of renal arterial pressure (RAP) in 4-week-old, anesthetized rats. Although mean arterial pressure in conscious SHRs (113±5 mmHg) and Wistar-Kyoto rats (110±6 mmHg) was not significantly different at 4 weeks of age, SHRs at 2 and 4 weeks of age already had increases in structurally based vascular resistance properties of ~30% above age- and weight-matched Wistar-Kyoto rats. Furthermore, the acute RAP-renal interstitial hydrostatic pressure relationship was found to be linear in both strains, and the temporal coupling of the stimulus to response was rapid; that is, renal interstitial hydrostatic pressure responses to changes in RAP were <2 s. Although the slope of the RAP-renal interstitial hydrostatic pressure relationship was not significantly different between strains, the relationship was significantly shifted (18%) to higher RAPs in SHRs. These results suggest that alterations in both vascular structure and renal function in young SHRs occur before elevations in mean arterial pressure. (Hypertension. 2012;59:979-984.)

Key Words: vascular resistance properties ■ renal hemodynamics ■ hydrostatic pressure ■ arterial pressure ■ pressure-natriuresis ■ SHR ■ WKY

Although the factors responsible for the initiation and development of genetic hypertension have been extensively studied in the spontaneously hypertensive rat (SHR), the basis and time course for blood pressure elevation remain enigmatic. Specifically, what remains controversial is whether the 2 most widely studied processes, vascular abnormalities and renal dysfunction, lead to increases in arterial pressure or vice versa.

Since the 1970s, it has been well established that vascular abnormalities, such as increases in vascular resistance, vascular reactivity to vasoconstrictors, and media:lumen ratio, are associated with hypertension in the adult SHR. Despite the number of studies describing vascular morphometric and histological differences in young SHRs, evidence linking blood pressure elevations with vascular structural and functional changes remains unresolved in the neonatal and weaning SHRs. The hypothesis that vascular structural changes antecedent and initiate a rise in arterial pressure has been disputed for 2 main reasons, the presence or lack of blood pressure elevation in studies of young SHRs and substantial differences between body weights of SHRs and Wistar-Kyoto (WKY) rats of blood pressure elevation in studies of young SHRs and substantial differences between body weights of SHRs and Wistar-Kyoto (WKY) rats or body weights not being reported. Since then, we reported longitudinally consistent differences in vascular resistance properties between weight-matched SHRs and WKY rats, where there was a 30% to 40% increase in SHRs throughout a broad interval of the postweaning period (ie, 4–50 weeks). However, it remains unknown whether similar differences in vascular resistance properties occur during the suckling neonatal (2 weeks) and weaning (3-4 weeks) periods, which represent a time of intensive vascular structural maturation and fall within the putative prehypertensive stage of SHRs.

Furthermore, given that the kidneys serve a critical role in the regulation of arterial pressure, it is not surprising that...
abnormalities specific to renal vascular resistance properties are associated with blood pressure elevations in young SHRs. Roman and Kaldunski demonstrated that components of the pressure-natriuresis mechanism (ie, medullary blood flow, urine flow, and sodium excretion) are shifted rightward toward greater renal perfusion pressures in 3- to 5-week-old SHRs. These findings suggest that renal medullary vascular resistance properties are elevated early in the development of hypertension and that alterations in medullary hemodynamics may participate in resetting pressure-natriuresis in young SHRs. Because transmission of renal arterial pressure (RAP) into the vasa recta capillaries in the renal medulla is a critical mediator of the pressure-natriuretic response and renal interstitial hydrostatic pressure (RIHP) is coupled to medullary blood flow, then a similar rightward shift is expected in the RIHP response to changes in RAP. Interestingly, we demonstrated recently, using a different approach, that the acute RAP-RIHP relationship has an underlying vascular basis. Specifically, the coupling between changes in RAP and RIHP was found to occur on a moment-to-moment basis and independent of neurohumoral control (ie, renin-angiotensin and autonomic nervous systems). Therefore, in addition to characterizing the pressure-natriuresis mechanism, this moment-to-moment relationship provides a hemodynamic assessment of renal medullary vascular resistance properties, where a rightward shift along the RAP operating point is indicative of increased renal vascular resistance. However, it remains unknown whether increases in renal medullary vascular resistance properties, and thereby pressure-natriuresis, occur in weaning SHRs (ie, 4 weeks old), at a time when renal organogenesis is complete.

We hypothesized that young SHRs are programmed to have increased vascular resistance properties and a rightward shift in the acute pressure-natriuresis mechanism before elevations in arterial pressure. Thus, the objective of the present study was to assess vascular resistance properties and the moment-to-moment RAP-RIHP relationship in weight-matched SHRs and WKY rats at 2 and/or 4 weeks of age.

**Methods**

**Animals**

Male WKY rats and SHRs aged 2 and 4 weeks old were used. Rats were housed individually (22±1°C; 12-hour light/dark cycle), with food and water provided ad libitum. All of the procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the Queen’s University Animal Care Committee. Details of the methods can be found in the online-only Data Supplement available at [http://www.hypertensionaha.org](http://www.hypertensionaha.org).

**Conscious Mean Arterial Pressure and Heart Rate Assessments**

Mean arterial pressure (MAP) was measured directly in 4-week-old WKY rats (n=6) and SHRs (n=7) surgically implanted previously (ie, 2–4 days) with aortic cannulas under ketamine (70 mg/kg IP) and xylazine (5–10 mg/kg IP) anesthesia. MAP and heart rate were calculated from average values recorded every 15 minutes for 3 hours after an hour acclimatization period starting at 9:00 AM.

**Hemodynamic Analysis of Hindlimb Vascular Resistance Properties**

Hindlimb vascular resistance assessments were performed in 2- and 4-week-old WKY rats and SHRs according to well-established methods that have been described previously. Briefly, perfusion pressures at maximum dilatation and maximum constriction were determined at a flow rate of 4 mL/min per 100 g of body weight. In addition, graded flow-pressure relationships were constructed at flow rates of 1, 2, 4, 6, and 8 mL/min per 100 g of body weight.

**In Vivo Assessments of Renal Medullary Vascular Resistance Properties**

In vivo assessments of renal medullary vascular resistance properties, specifically the RAP-RIHP relationship, were performed in anesthetized 4-week-old WKY rats (n=11) and SHRs (n=9), based on methodology described previously.

**Statistical Analysis**

All of the statistical calculations were performed and graphs constructed using Prism 5 (GraphPad Software). Linear regression analysis was used to calculate the slopes and y intercepts of the hindlimb flow-pressure and RAP-RIHP relationships. The Grubb test was conducted on all of the data sets to determine statistical outliers. All of the data are presented as mean±SEM. Statistical significance between WKY rats and SHRs was determined using a Student t test, and P<0.05 was considered statistically significant.

**Results**

**Hindlimb Vascular Resistance Assessments in 2- and 4-Week-Old Rats**

**Physical and Hemodynamic Characteristics**

The growth curves of the SHRs and WKY rats used have been shown previously to be almost identical. Corroborating previous findings, in the present study, rats had similar body weights at the predetermined ages of 2 weeks (SHR, 24.0±0.2 g; WKY, 24.7±0.3 g) and 4 weeks (SHR, 66.9±0.8 g; WKY, 68.0±1.0 g) without any special selection of animals. In addition, we determined that SHRs and WKY rats of 1 to 3 weeks of age have similar hindlimb weights. At 7 to 10 days, hindlimb weights were 18% of body weight and at 28 days, 26% of body weight (data not shown). Previous data has shown that SHRs, in comparison with WKY rats, obtained from this colony have similar proportional hindlimb weights throughout their life span. Furthermore, at 4 weeks of age, there was no significant difference in conscious MAPs (SHR, 113.0±5.0 mm Hg; WKY, 110.2±5.9 mm Hg), although heart rate was elevated by 16% in the SHRs (459.1±9.5 bpm) versus WKY rats (395.2±7.8 bpm; P<0.05).

**Resistance Properties of the Neonatal Hindlimb Vasculature**

Perfusion pressure at maximum dilatation was elevated by ~30% in SHRs at both 2 and 4 weeks of age (P<0.05; Figure 1A). A similar increase in absolute perfusion pressures at maximum dilatation between 2 and 4 weeks was found in both SHRs and WKY rats, in accordance with the rapid normal maturation during this time (Figure 1A). The pressure responses to acute changes in flow in the vasculature of SHRs at 2 and 4 weeks of age were significantly elevated in comparison with age-matched WKY rats at all of the flow...
maximum pressure response with supramaximal constrictor mixture. The inability to produce a maximum pressure response using methoxamine alone in both SHRs and WKY rats was 80% to 90% of the total maximum pressure response observed with the supramaximal constrictor mixture. The inability to produce a maximum pressure response with \( \alpha_1 \)-adrenoceptor activation alone was similar at both ages.

![Diagram A](image1.png)

Figure 1. A. Perfusion pressure at maximum dilatation (ppMD) produced by infusing papaverine at a flow rate of 4 mL/min per 100 g of body weight (BW), (B) flow-pressure relationships at maximum dilatation, and (C) perfusion pressure at maximum constriction (ppMC) produced by infusing supramaximal concentrations of angiotensin II, vasopressin, and methoxamine at a flow rate of 4 mL/min per 100 g of BW, in weight-matched pairs of spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) at 2 (left) and 4 (right) weeks of age. *\( P<0.05 \) vs WKY and †\( P <0.05 \) vs elevations (y intercepts) of WKY flow-pressure lines.

In Vivo Assessments of Renal Medullary Vascular Resistance Properties of 4-Week-Old Rats

Physical and Hemodynamic Characteristics

Body weights of 4-week-old SHRs and WKY rats used were not significantly different (Table) and did not significantly differ from age-matched rats used in the hindlimb vascular resistance assessments. Consistent with the development of hypertension, kidney weights, kidney:body weight ratios, and the left ventricular:body weight ratios were significantly higher in SHRs than in WKY rats (\( P<0.05 \); Table).

In Vivo Renal Hemodynamic Properties of Neonatal Rats

Mean RAP was \( \approx 18\% \) greater in anesthetized SHRs compared with age-matched WKY rats (\( P<0.05 \)), whereas RIHP was not different (Table). As a result, there was a rightward shift in the acute RAP-RIHP relationship of SHRs in comparison with WKY rats (\( P<0.05 \); Figure 2). Furthermore, there were no differences in the slopes of the overall acute RAP-RIHP relationship between the 2 strains of rats (SHR, 2.02 mmHg; WKY, 3.6 mmHg). The overall time for RIHP to respond to any type of change in RAP in the SHR was 2 times quicker than in the WKY rat (\( P<0.05 \)). A similar trend between the 2 strains was present when comparing pressor versus depressor times.

Interestingly, pressor manipulations in RAP resulted in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAP, mm Hg†</td>
<td>72.76±3.84*</td>
<td>86.14±4.57*</td>
</tr>
<tr>
<td>RIHP, mm Hg†</td>
<td>3.13±0.59</td>
<td>3.03±0.48</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>47.78±0.54</td>
<td>48.90±0.93</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>70.26±1.10</td>
<td>68.03±0.75</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>0.37±0.02*</td>
<td>0.46±0.004*</td>
</tr>
<tr>
<td>Right kidney:body wt</td>
<td>0.005±0.0003*</td>
<td>0.007±0.0001*</td>
</tr>
<tr>
<td>Left ventricle weight, g</td>
<td>0.22±0.01</td>
<td>0.23±0.004</td>
</tr>
<tr>
<td>Left ventricle:body wt</td>
<td>0.0031±0.0001*</td>
<td>0.0033±0.0001*</td>
</tr>
<tr>
<td>Right ventricle weight, g</td>
<td>0.07±0.004</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>Right ventricle:body wt</td>
<td>0.0009±0.0001</td>
<td>0.0009±0.0001</td>
</tr>
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RAP indicates renal arterial pressure; RIHP, renal interstitial hydrostatic pressure; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat. Values are mean±SEM. *\( P<0.05 \) vs WKY rat. †Data were measured under anesthesia.
The acute renal arterial pressure (RAP)-renal interstitial hydrostatic pressure (RIHP) relationship of 4-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Linear regression analysis revealed a significant difference in the elevations (y-intercepts) of the SHR and WKY renal hemodynamic relationships (P<0.05 vs WKY) and the slopes of the pressor (●) versus depressor (□) relationships (tP<0.05 vs depressor). There was also a significant difference in RAP at all similar levels of RIHP (tP<0.05 vs WKY). Data are displayed in bins of 10 mm Hg for RAP manipulations of ±30 mm Hg around the operating point. Values are mean±SEM.

Figure 2. The acute renal arterial pressure (RAP)-renal interstitial hydrostatic pressure (RIHP) relationship of 4-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). Linear regression analysis revealed a significant difference in the elevations (y-intercepts) of the SHR and WKY renal hemodynamic relationships (P<0.05 vs WKY) and the slopes of the pressor (●) versus depressor (□) relationships (tP<0.05 vs depressor). There was also a significant difference in RAP at all similar levels of RIHP (tP<0.05 vs WKY). Data are displayed in bins of 10 mm Hg for RAP manipulations of ±30 mm Hg around the operating point. Values are mean±SEM.

Figure 3. Time delay for renal interstitial hydrostatic pressure (RIHP) to respond to a change in renal arterial pressure (RAP) in Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) at 4 weeks of age. tP<0.05 vs depressor RAP manipulations of 30 mmHg in both WKY rats and SHRs; *P<0.05 vs WKY at same magnitude of RAP manipulation. Data are displayed in bins of 10 mm Hg for RAP manipulations of ±30 mmHg. Values are mean±SEM.

Discussion

The major findings of the present study were as follows: (1) vascular resistance properties are higher in weight-matched SHRs compared with WKY rats at both 2 and 4 weeks of age, suggesting that vascular abnormalities are present before differences in conscious MAP between both strains; (2) the acute RAP-RIHP relationship is shifted toward greater pressures in 4-week-old, weight-matched SHRs than WKY rats; and (3) the time delay for RIHP responses to acute changes in RAP is <2 s, indicating that there is an underlying vascular basis for this component of the pressure-natriuresis mechanism. Together, these findings suggest that neonatal and weaning SHRs have phenotypic differences in their vasculature and renal hemodynamics compared with age-matched WKY rats. Consequently, these phenotypic differences could, in part, render them susceptible to hypertension later in adulthood.

There has been a long-standing debate about whether elevations in arterial pressure precede alterations in vascular structure or vice versa.4–8,10,11,15,18 In our colony, conscious MAP was not different between SHRs and WKY rats at 4 weeks of age; however, structurally based vascular resistance properties were higher in both 2- and 4-week-old SHRs versus WKY rats. These findings extend our previous results in rats of the same colony and suggest that vascular resistance properties differ to the same extent (ie, ~30%) from neonatal to adult SHRs (~50 weeks), whereas the full expression of hypertension in SHRs only appears at ~14 weeks of age.9 That is, because the full magnitude of the difference in SHR versus WKY vasculature already exists at 2 weeks of age and there does not appear to be a critical period between 2 and 50 weeks during which SHR vessels function differently, these data do not support the concept that elevated arterial pressure is a predominant factor in inducing further vascular structural and functional adaptation.

It is important that evidence for a prevailing difference in the configuration of blood vessel architecture in these neonatal SHRs and WKY rats comes from 3 assessments. First, differences in perfusion pressures at maximum dilatation in hindlimb vasculature of SHRs elevate pressure ~30% more than the normotensive rat. According to the Poiseuille law, which states that resistance to flow is inversely proportional to radius to the fourth power, it can be calculated that, in the average hindlimb blood vessel of ~2–4, or even 50-week-old SHRs,9 the average lumen is ~7% smaller than in WKY rats either when there is no vasoconstrictor tone or at the same level of vessel contraction, that is, structurally determined. Second, assessments of perfusion pressure at different flow rates extend this concept further, where at all flow rates between 1 and 8 mL/min per 100 g of body weight, resistance to flow is elevated such that there is a narrowing of the vessel lumen that is not dependent on the distending pressure. Last, perfusion pressures maximum constriction in hindlimb vascular beds of young SHRs demonstrate a greater contractile capacity compared with WKY rats. Mulvany et al2 have demonstrated differences in the contractile capacity of small isolated segments of resistance vessels in SHRs that can be wholly accounted for by an increased muscle mass within the blood vessel wall. Accordingly, the increase in resistance/vasoconstrictor capacity of ~30% suggests that the average hindlimb blood vessel of the neonatal SHRs relative to WKY rats has a smaller lumen and/or a thicker wall.

Similar to vascular resistance properties of peripheral resistance vessels (ie, hindlimb arteries), previous findings demonstrated that renal vascular resistance is elevated in young "prehypertensive" SHRs (ie, ≤4 weeks of age)22–26 and continues to be elevated into adulthood (ie, 16 weeks of age).22,23,25,26 Given that transmission of RAP to vasa recta...
capillaries in the renal medulla is a critical mediator of the pressure-natriuretic response, an elevated renal vascular resistance would require a greater RAP to induce homeostatically appropriate changes in sodium excretion.\textsuperscript{21,36} Corroborating previous findings,\textsuperscript{27,28} the present study demonstrated that, although the overall, pressor, and depressor slopes of the acute RAP-RIHP relationship are not different between strains, there is a rightward shift toward a greater RAP in 4-week–old SHRs relative to WKY rats. This finding not only supports the proposed cascade of players in pressure-natriuresis (ie, medullary blood flow, RIHP, urine flow, and sodium excretion)\textsuperscript{37} but also, more importantly, the hypothesis that renal medullary vascular resistance properties are elevated in young SHRs\textsuperscript{28,34} after completion of postnatal renal organogenesis (ie, postnatal day 30).\textsuperscript{28,34} This notion is further supported by the moment-to-moment nature of RIHP responses (ie, \textless{} 2 s) to changes in RAP in 4-week–old rats. Specifically, it appears that there is an underlying vascular basis for this component of the pressure-natriuresis mechanism, because it is unlikely that slower-acting neurohumoral mechanisms play a role in altering the acute functioning of this relationship.\textsuperscript{31} Thus, renal medullary vascular resistance properties appear to be elevated in weaning SHRs and may participate in the development of hypertension by inducing a rightward shift in the acute pressure-natriuresis mechanism. It should, however, be noted that other factors (eg, renal capsule or pressor versus depressor stimuli) may also, in part, influence this acute relationship, as well as the full expression of MAP, and thereby warrant further investigation (a post hoc assessment and detailed discussion on these factors can be found in the online-only Data Supplement).

Despite the rightward shift in the moment-to-moment RAP-RIHP relationship, conscious MAP was found to not be different between weight-matched SHRs and WKY rats at 4 weeks of age. One explanation for this paradoxical finding may be that young SHRs are in a state of sodium and/or volume retention. It may be that, during development, these young animals are in a dynamic state of cardiovascular flux, where various sodium/fluid body homeostatic mechanisms modulate the overall pressure-natriuretic response to maintain a positive sodium balance necessary for growth.\textsuperscript{38,39} Indeed, it has been reported previously that young SHRs retain 4-fold more sodium (ie, at 4–5 weeks of age)\textsuperscript{38} and have lower fractional sodium and water excretion levels (ie, during the second postnatal month)\textsuperscript{40} than WKY rats despite having similar intakes. Although not assessed in the present study, the rightward shift in the moment-to-moment RAP-RIHP relationship coupled with no differences in MAP support the notion that young SHRs may be in a relatively greater state of sodium retention than WKY rats. The exact reasons for this are not completely known; however, it can be speculated that disorders in sodium homeostatic mechanisms (eg, renin-angiotensin and sympathetic nervous systems),\textsuperscript{41} changes in renal interstitial compliance,\textsuperscript{42} and a relatively higher positive sodium balance requirement for growth than that of WKY\textsuperscript{38,39} may influence the overall pressure-natriuretic response in young SHRs without influencing MAP. Interestingly, as the animals mature and a positive sodium balance is no longer necessary for growth, the differences in sodium balance diminish between SHRs and WKY rats, where by postnatal week 9 through adulthood (ie, at the time hypertension becomes evident in the SHR), no differences in sodium balance exist.\textsuperscript{40,41} Although the outcomes of these studies provide a better understanding of vascular structure and renal hemodynamics in young SHRs versus WKY rats, there are some limitations that need to be addressed. First, the presence of potential unanticipated or uncontrollable environmental factors during critical periods of the perinatal development may have influenced the programming of the phenotypes expressed in these rats. Furthermore, comparisons of hindlimb and renal hemodynamic assessments might be complicated, because these studies were conducted in 2 separate colonies; however, the rats had similar body weights, as well as left ventricular: body weight ratios, suggesting that the growth rates of these 2 colonies are comparable.\textsuperscript{9} Lastly, rats had to be anesthetized for the renal hemodynamic assessments. Given that it has been demonstrated previously that SHRs may have altered sensitivity to certain anesthetics compared with WKY rats, this may have affected the outcome.\textsuperscript{43} However, despite this caveat, the results of the present study are comparable to those of others.\textsuperscript{27,28}

Taken together, these data indicate that the SHR may be programmed susceptible to developing hypertension via a combination of increased structurally based vascular resistance properties and a rightward shift in acute renal vascular properties after critical periods of perinatal development, that is, vasculogenesis and renal organogenesis, respectively.

**Perspectives**
The present studies suggest that there is a probable mechanistic and temporal link between alterations in vascular structure and renal function in SHRs, which make these animals susceptible to developing hypertension in adult life. Future studies are warranted to investigate the genetic and environmental mechanisms “programming” these vasculogenic alterations in young and growing SHRs. Such knowledge could lead to the development of interventions during critical periods of perinatal programming aimed at improving blood pressure outcomes in adult life.

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**Disclosures**
None.

**References**


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Altered Vascular Resistance Properties and Acute Pressure-Natriuresis Mechanism in Neonatal and Weaning Spontaneously Hypertensive Rats

Marina Komolova¹, Peter Friberg², and Michael A. Adams¹

¹Department of Biomedical & Molecular Sciences, Queen’s University, Kingston, ON, Canada
²Department of Clinical Physiology, University of Göteborg, Sahlgrenska University Hospital, Sweden

Correspondence to:
Dr. Michael A. Adams
Department of Biomedical & Molecular Sciences
Queen’s University
Kingston, Ontario, Canada, K7L3N6
Telephone: 613-533-6516
Fax: 613-533-6412
Email: adams@queensu.ca
Methods:

Animals

Male SHR and WKY (12-14d and 28d of age) obtained from the animal care facility of the Baker Medical Research Institute, Melbourne, Australia (colonies originating from a breeding nucleus provided by Dr. Y. Yamori, Japan) were housed in group cages either with their mother (2 weeks) or with their male littermates (4 weeks). These rats were used for the conscious cardiovascular profiling and hindlimb vascular resistance assessments.

Male SHR and WKY (21d of age; littermates) were obtained from Charles River Laboratories (Montreal, QC), and were acclimatized for 7d prior to experimentation. These rats were used for in vivo renal hemodynamic assessments.

Both colonies of SHR and WKY had a similar age-body weight relationship with the result bearing that no special pre-selection of rats was required to obtain age and weight matched pairs. Rats were housed individually (22±1ºC; 12h light/dark cycle), with food and water provided ad libitum. All procedures followed guidelines of the Canadian Council on Animal Care and were approved by the Queen’s University Animal Care Committee.

Hemodynamic Analysis of Hindlimb Vascular Resistance Properties

SHR and WKY at 2 and 4 weeks of age were anesthetized with sodium pentobarbital (60mg/kg, IP) and the lower abdominal aorta was exposed at the iliac bifurcation through a midline incision under microscopic examination. The middle caudal and the caudal mesenteric arteries were ligated and the rats heparinized (1000IU/kg, IV) prior to cannulation of the aorta using either a 25 or 23 gauge needle proximal to the bifurcation. Perfusion was started immediately after the spinal cord and vena cava were transected proximal to the cannula entrance. The perfusate (Tyrode-Dextran) and dual peristaltic pump delivery system was identical to that described previously. Approximately 2-3min after the start of perfusion (1-2ml/min/100 g of body weight) and after the hindlimb vessels were cleared of blood perfusate containing papaverine-HCL (1.5mg/kg) was given for several minutes to ensure maximum dilatation. Some 20min after washing out papaverine, perfusion pressure was measured at maximum dilatation (ppMD) in rats in which the pressure had remained constant over the preceding washout interval, thereby excluding edema formation. At this point a graded flow-pressure relationship was constructed (1, 2, 4, 6 and 8ml/min/100 g of body weight) to assess differences in flow-pressure responses of neonatal and weaning SHR and WKY, as calculated from the slope of this line. Subsequently, maximum constrictor (ppMC) responses were obtained after an α1-adrenoceptor agonist, methoxamine (200µg/ml) was given alone and then after a cocktail containing supra-maximal concentrations of angiotensin II (20µg/ml) and vasopressin (2IU/ml).

Additionally, three series of experiments were performed to attempt to assess hindlimb vascular resistance properties in 6-8d old SHR and WKY weighing between 5-8g; however, these attempts proved to be unsuccessful. Insertion of the small Teflon tubing into the aorta induced a rapid disintegration of the vascular wall. The marked difference even between the first and second post-natal week with respect to catheter insertion reveals the degree of immaturity of the structural elements of the aorta in 1 week old rats making it not technically feasible to conduct these experiments.
**In Vivo Assessments of Renal Medullary Vascular Resistance Properties**

SHR (n=9) and WKY (n=11) rats at 4 weeks of age were anesthetized with ketamine (30mg/kg, IP) and Inactin (thiobarbital sodium; 100mg/kg, IP). Body temperature was monitored using a thermistor (model 402; Yellow Springs Instruments) and maintained at 37±0.5°C using a temperature controller (model 73A; Yellow Springs Instruments) connected to a heating pad and lamp. Additional anesthetic was given, as necessary, throughout the experiment. The rats were tracheostomized (PE-90) and 95% O₂ / 5% CO₂ was passively blown over the intake to assist respiration (i.e. the source was not directly attached to cannula). A midline abdominal incision was made, and the right kidney was removed. A saline-filled catheter was introduced into the inferior vena cava (at the level of the right iliolumbar vein; secured with cyanoacrylate glue) for continuous infusion of saline (0.9% NaCl) at 33μl/min/100g of body weight via a syringe pump (KD Scientific 220 Multi-Syringe Pump; Fisher Scientific) to compensate for fluid loss during surgery.

In vivo assessment of renal medullary vascular resistance properties was based on methods described previously². The superior mesenteric artery was catheterized with heparinized saline-filled (50IU/ml) polyethylene tubing (PE-10) for continuous measurement of arterial pressure at the level of the renal artery (hereinafter referred to as RAP). A silastic balloon cuff was placed between the celiac and superior mesenteric arteries and another silastic balloon cuff was placed just below the left renal artery. The silastic balloon cuffs allowed for manipulation and control of RAP over a wide range of pressures (i.e. pressor and depressor). An electrosurgical unit (Elmed ESU 30; Elmed Incorporated) was used to create a 1.5mm-hole into the longitudinal axis of the left kidney for insertion of a catheter for RIHP measurements (PE-50 tubing fitted with 1.5mm long polyethylene matrix of 15-45μm pore size; Porex). The catheter was pre-flushed and pre-filled with heparinized saline (50IU/ml) and then held in place with cyanoacrylate glue. RAP and RIHP were continuously monitored via pressure transducers (model CDX3, Cobe) connected to a PowerLab/8s (ADInstruments) data acquisition system with Chart v. 4.2.2 software.

After surgery, an equilibration period of ~15min was allowed prior to recording steady state baselines of RAP and RIHP. The acute RAP-RIHP relationship was determined via short-term manipulations of RAP (1-60s) using the silastic balloon cuffs in encapsulated kidneys. These manipulations consisted of sequential pressor and depressor changes in RAP of various magnitudes (±30mmHg from baseline). Upon completion of the experiment, the kidneys were decapsulated, and after ~5min, baseline RAP and RIHP were recorded. Then, hematocrit was determined and the rats were euthanized via excision of the heart. Lastly, the left kidney was removed, and the position of the RIHP catheter was verified to be at the cortical-outer medullary junction.

Baseline values for RAP and RIHP were determined by averaging the 60s period prior to starting RAP manipulations. The overall, pressor, and depressor slopes of the acute RAP-RIHP relationship were determined from steady-state values of RAP and RIHP following short-term manipulations of RAP. The time delay between the initial RAP change and the onset of RIHP response was characterized as the difference between the first detectable change in RAP and RIHP from the operating point following a manipulation.
Discussion:

*Acute RAP-RIHP relationship: Effects of the pressor versus depressor stimuli in weaning rats*

In an animal model of salt-sensitivity and hypertension (i.e. atrial natriuretic peptide knockout mouse), we previously demonstrated that the acute RAP-RIHP relationship responds differentially to pressor versus depressor stimuli\(^3\). Specifically, in addition to a rightward shift towards greater RAP (i.e. indicative of greater structurally-based vascular resistance properties), there was also a blunting of the pressor slope (i.e. indicative of salt-sensitivity)\(^4\). Herein, young SHR display a parallel shift in both pressor and depressor components to a greater RAP, and interestingly, in *both* SHR and WKY, the pressor slope is steeper than the depressor. The role for this differential response to pressor versus depressor stimuli is not fully understood and requires further investigation; however, given that there is no change in slope in both strains, this suggests that these animals are not likely salt-sensitive at 4 weeks of age. Furthermore, the time delay for the initial RIHP response to a pressure change is markedly shorter following pressor stimuli, indicating that these rats are not salt-sensitive, since elevations in RAP are dealt with more efficiently than decreases.

*Acute RAP-RIHP relationship: Effects of the renal capsule in weaning rats*

The maintenance of RIHP in young SHR to comparable levels of WKY does not only depend on the level of RAP, but also the function of the renal capsule. The capsule aids in the distribution of hydrostatic pressure generated by MBF throughout the renal interstitium\(^5,6\). Decapsulation has been shown to blunt RIHP in adult normotensive rats\(^5\); however it has never been investigated in young rats. For the first time, the present study demonstrates that decapsulation in young SHR results in a marked blunting of baseline RIHP, whereas WKY remain unaffected (Figure S1). This suggests that following completion of renal organogenesis, the renal capsule of SHR is programmed such that it aids in maintaining similar basal RIHP levels to WKY. This may also explain why the initial RIHP response time following RAP changes in encapsulated kidneys is quicker in the SHR than WKY; however this speculation requires further investigation (Figure S1). Interestingly, by adulthood, the capsule appears to lose its function in SHR, and conversely becomes necessary in WKY for the maintenance of RIHP\(^7\). Consequently, RIHP is blunted in adult SHR, and the natriuretic response is no longer sensitive to decapsulation, indicating that the animal is salt-sensitive (i.e. blunted slope of RAP-RIHP relationship)\(^4,7\). Together, it can be speculated that the newly developed kidneys of young SHR maintain sodium and fluid homeostasis, in part, due to a less elastic or tougher capsule, and by adulthood, these properties are abolished leading to salt-sensitivity.

It should also be noted that young SHR exhibit similar characteristics to animals that are in a cardiovascular state of flux, such as during pregnancy. It has been previously demonstrated by Khraibi\(^8\) that changes in the RAP-RIHP relationship and pressure-natriuresis mechanism (i.e. sodium excretion) can occur without any significant changes to MAP, particularly in pregnant rats, as a result of alterations in renal interstitial compliance (i.e. changes in the interstitial matrix or changes in the stiffness characteristics of the renal capsule). Interestingly, as mentioned above, we also found differences in the effects of the renal capsule on the moment-to-moment RAP-RIHP relationship between 4 week old SHR and WKY, where the renal capsule appeared to contribute to the basal levels of RIHP in SHR. This is not surprising, given that these young
animals are rapidly growing and developing, and thereby are also in a state of cardiovascular flux. Thus, further investigation into this matter is warranted to clarify the role of renal capsule in young SHR during development.
References:


**Figure S1**: Effects of renal decapsulation on percent RIHP of RAP at baseline in WKY and SHR at 4 weeks of age. A post-hoc comparison of percent RIHP of RAP in encapsulated versus decapsulated kidneys revealed that decapsulation decreased percent RIHP of RAP only in SHR, indicating that the renal capsule may play a more prominent role in maintaining RIHP in SHR. *P<0.05 vs. encapsulated SHR kidney. Values are mean ± SEM.