Role of Afferent Renal Nerves in Spontaneous Hypertension in Rats

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In the present study we examined sympathetic function and baroreceptor reflex sensitivity in adult spontaneously hypertensive rats (SHR) after a selective transection of afferent renal nerves in the prehypertensive and established phases of hypertension. Renal deafferentation performed between 3 and 4 weeks after birth did not influence the course of the development of high blood pressure when compared with sham-operated rats. Mean arterial pressure, heart rate, and plasma norepinephrine concentrations were similar in both groups when measured at 13 weeks after renal deafferentation. However, blood pressure responses to ganglionic blockade with hexamethonium were significantly reduced in the renal deafferented SHR. Baroreceptor reflex sensitivity, assessed by heart rate responses to blood pressure changes induced by phenylephrine and nitroprusside, was significantly enhanced in these rats. When renal deafferentation was performed in adult SHR with established hypertension, mean arterial pressure decreased slightly but significantly by 5%. Heart rate, plasma norepinephrine concentrations, and responses to hexamethonium were not affected by this procedure. However, in the renal deafferented adult SHR, heart rate responses to phenylephrine but not to nitroprusside were significantly increased. Thus, in contrast to efferent renal nerves, afferent renal nerves do not play an important role in the development and maintenance of high blood pressure in SHR, but may contribute to the mechanisms that alter sympathetic function and baroreceptor reflex sensitivity in SHR during the development of hypertension. (Hypertension 1989;13:327-333)

Evidence has accumulated that the development of spontaneous hypertension in rats is characterized by an elevated sympathetic nervous system activity as measured by direct nerve recordings, responses to ganglionic blockade, or by enzymatic measurements. The cause for this sympathetic abnormality is as yet unknown.

Central as well as peripheral nervous factors, especially the renal nerves, have been suggested to contribute to the initiation of spontaneous hypertension. Lesions of various brain structures decrease blood pressure in spontaneously hypertensive rats (SHR), probably via a reduction in central sympathetic nervous activity. Hypotensive responses have also been observed after bilateral renal denervation in these rats. The antihypertensive mechanisms of renal denervation are still unclear. Renal denervation may lead to a hypotensive effect through alterations in renal excretory function or renin release. In addition, renal denervation changes hypothalamic norepinephrine content, cardiovascular responses to ganglionic blockade, and electrical hypothalamic stimulation in several experimental hypertensive models. These data suggest that peripheral section of these nerves may alter central sympathetic nervous activity.

In the previously mentioned reports, renal denervation techniques did not discriminate between efferent renal nerve (ERN) and afferent renal nerve (ARN) fibers. This implies that the centrally mediated hypotensive effects of renal denervation can be explained by changes in renin secretion and plasma angiotensin II levels as a result of a decreased ERN activity. Alternatively, these effects may be mediated by the interruption of ARN. Histological and electrophysiological studies have shown that ARN project to structures within the central nervous system that are involved in cardiovascular regulation. Stimulation of ARN in conscious animals results in a sympathetically mediated pressor response and may lead to hypertension. In a previous report, we have shown that rats in which a selective denervation of afferent renal nerves (ARN-
x) was performed at 4 weeks of age had similar basal 
blood pressures (measured via indwelling arterial 
catheters) as compared with sham-operated control 
rats. However, systolic blood pressure, as measured 
by the tail-cuff method, was significantly reduced in 
these ARN-x SHR. This suggested an impaired abil-
ity of ARN-x SHR to respond to stress. Fur-
more, electrophysiological studies have shown that 
ARN in SHR are hyperresponsive to certain stimuli 
compared with the response evoked in normotensive 
control rats, although contrasting results have been 
reported elsewhere.

Therefore, the present study was designed to test 
whether the ARN contribute to sympathetic func-
tion in SHR. As an index for this, baroreceptor 
reflex sensitivity and responses to ganglionic block-
ade were measured in conscious intact and ARN-x 
rats. To compare developmental and direct effects, 
ARN-x was performed in prehypertensive and adult 
hypertensive rats.

Materials and Methods

Experiments were performed in SHR and Wistar-
Kyoto (WKY) normotensive control rats from our 
own breeding colonies. Originally the rats were 
derived from the Okamoto-Wistar strain. During 
the experiments, the animals were housed under 
standard laboratory conditions (constant tempera-
ture 21°C, a 12-hour light/dark cycle, standard rat 
food, and tap water ad libitum).

Experiment 1: Effects of Renal Deafferentation in Prehypertensive Spontaneously Hypertensive Rats

Young female SHR (SHR_y) were equally 
divided between two groups. In one group of rats 
(23-27 days old) a selective renal deafferentation 
of the right kidney was performed by unilateral section 
of the right dorsal roots of the vertebrae T8-L1. 
This procedure has been described in detail 
elsewhere. The second group of rats served as 
sham-operated controls. These animals underwent 
the same surgical procedures, except for the actual 
rhizotomy. After 2-3 days of recovery, all rats were 
uninephrectomized (left kidney) under light ether 
anesthesia to exclude a possible role of the contra-
lateral kidney. Systolic blood pressure was 
measured between 9:00 and 12:00 AM at weekly 
intervals from the third through the 15th week, using 
the tail-cuff method. Heart rate was derived from the 
arterial pulse signal with a tachograph (Narco-
biology Systems, Houston, Texas). During this period 
(week 10) rats were placed in metabolic cages (type 
1760, Techniplast, Buggugiate, Italy) for 7 days. 
After 4 days of habituation, daily intake of food and 
water was monitored for 3 days. Urine collections 
(24 hour) were made for determination of urinary 
sodium excretion, measured by flame spectropho-
tometry. When blood pressure had reached a con-
stant level in the sham-operated SHR (week 16), 
catheters were implanted in all rats to perform an 
experimental protocol as described below. Briefly, 
under ether anesthesia the right suprarenal artery 
was cannulated with a stretched PE-10 catheter for 
intrarenal infusions. The catheter was attached to 
an osmotic minipump (Alzet type 2001, Alza Cor-
poration, Palo Alto, California) filled with saline for 
constant perfusion (1 µl/hr). After a 3-day recovery 
period, rats were again anesthetized with ether, and 
catheters (PE-10) were inserted via the femoral 
vessels into the caval vein for intravenous infusions 
and the abdominal aorta for direct blood pressure 
measurements. These catheters plus the catheter 
for intrarenal infusions were brought subcutane-
ously to the back of the neck, anchored, exterior-
ized, and filled with 0.9% NaCl. The osmotic mini-
pump was detached, and the catheter for intrarenal 
infusion was filled with heparinized (5 IU/ml) saline 
and closed with a metal plug.

Experiment 2: Effects of Renal Deafferentation in Adult Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

Male 16-18-week-old SHR (SHR_adult) and WKY 
rats (WKY_adult) were nephrectomized on the left 
side 4-5 weeks before entering the study. To deter-
mine the effect of ARN-x, both SHR and WKY rats 
were divided into two groups with comparable 
mean blood pressures, as measured with the tail-cuff 
method before surgery. These animals underwent 
surgery for intrarenal infusions. The catheter was attached to 
an osmotic minipump (Alzet type 2001, Alza Cor-
poration, Palo Alto, California) filled with saline for 
constant perfusion (1 µl/hr). After a 3-day recovery 
period, rats were again anesthetized with ether, and 
catheters (PE-10) were inserted via the femoral 
vessels into the caval vein for intravenous infusions 
and the abdominal aorta for direct blood pressure 
measurements. These catheters plus the catheter 
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ously to the back of the neck, anchored, exterior-
ized, and filled with 0.9% NaCl. The osmotic mini-
pump was detached, and the catheter for intrarenal 
infusion was filled with heparinized (5 IU/ml) saline 
and closed with a metal plug.

Experimental Protocol

Before the following pharmacological experi-
ments were performed, baseline (30-60 minutes) 
mean arterial pressure and heart rate were recorded 
with a pressure transducer (CP-01, CTC, Ingle-
wood, California) in connection with a tachograph.

Verification of renal deafferentation (day 1). Pre-
vious studies have shown that intrarenal infusion of 
bradykinin causes an increase of mean arterial 
pressure and heart rate through activation of ARN. 
This reflex can be prevented by ARN-x. Thus, 
to test the functional integrity of the ARN, rats 
were given 5 minutes intrarenal and intravenous 
infusions of bradykinin (0.3 and 1.0 µg/min of 
triacetate salt) in random order at 15-minute inter-
vals. Mean (2-5 minute) changes in mean arterial 
pressure and heart rate were calculated.

Measurement of baroreceptor reflex sensitivity 
(day 2). Baroreceptor reflex sensitivity was deter-
mined as described by Smits et al. As an index for 
baroreceptor reflex sensitivity of heart rate the ratio 
was calculated between changes in heart period 
(HP=60.000/HR, where HP is heart period and HR 
is heart rate) and changes in mean arterial pressure 
induced by intravenous bolus injections of phenyl-
ephrine (Sigma Chemical Co., St. Louis, Missouri) and nitroprusside sodium (Hoffmann-La Roche, Basel, Switzerland) administered in random order. At least nine different depressor and nine different pressor responses were induced, ranging between -50 to 0 and 0 to 60 mm Hg, respectively. Heart rate varied between 200 and 500 beats/min. Only linear regression lines with a correlation coefficient greater than 0.80 and a p<0.05 were included.

Ganglionic blockade (day 3). To assess the contribution of the sympathetic nervous system tone to the maintenance of blood pressure in these conscious animals, changes in mean arterial pressure and heart rate were recorded (mean of 5-15 minutes) after intravenous injection of 20 mg/kg hexamethonium (Sigma Chemical Co.), which causes total ganglionic blockade in rats.2

Plasma and tissue norepinephrine determination. Before any drugs were given (day 1) a 0.5 ml arterial blood sample was taken from all rats for determination of plasma norepinephrine levels. Plasma norepinephrine concentration was measured with high-performance liquid chromatography (see Reference 30) in combination with coulochemic detection (Coulochem 5200 A, ESA, Bedford, Massachusetts).

At the end of the experiments, the kidney of the adult SHR was extirpated, weighed, and homogenized and then assessed for norepinephrine content by high performance liquid chromatography. Rats were then perfused transcardially with 10% formaldehyde (about 90% of the ARN31) were included in the data analysis. In sham-operated ARN-x rats all dorsal roots were intact.

Statistics
All data are expressed as mean±SEM unless stated otherwise. Data on the development of systolic blood pressure and heart rate were analyzed by means of an analysis of variance (ANOVA) for growth curves.32 Mean arterial pressure and heart rate responses to bradykinin infusions were analyzed by two-way ANOVA. Results from the study on baroreceptor reflex sensitivity and responses to hexamethonium between intact and ARN-x rats were analyzed with Student's t test. Differences in these variables between SHR and WKY rats were analyzed by ANOVA. Statistical significance was accepted at p<0.05.

Results
Effects of Renal Deafferentation in Spontaneously Hypertensive Rats
Up to the 10th week, blood pressure development curves (see Reference 22) were similar in ARN-x and sham-operated SHRyoung. From 12 weeks on, systolic blood pressure was slightly (10-15 mm Hg) but significantly (p<0.05) reduced in the ARN-x SHRyoung. No differences in heart rate or body weight between the two groups were observed during the whole period (4-16 weeks). Values for 24-hour food and water intake and urinary volume and sodium excretion 7 weeks after ARN-x are presented in Table 1. No statistical differences were found in any parameter between both groups.

Direct Blood Pressure Measurements
Basal mean arterial pressure, heart rate (mean values over 3 days), and plasma norepinephrine levels of all rats studied in this experiment are shown in Table 2. Although systolic blood pressure in the developmental study differed in the sham-operated and renal deafferented Wistar-Kyoto rats and spontaneously hypertensive rats.

### Table 1. Effects of Renal Deafferentation in Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Food intake (g)</th>
<th>Water intake (ml)</th>
<th>Uv (ml)</th>
<th>Unore+ (meq)</th>
<th>Uk+ (meq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated SHR</td>
<td>16.9±0.6</td>
<td>32.0±3.2</td>
<td>17.5±0.9</td>
<td>3.29±0.26</td>
<td>2.48±0.28</td>
</tr>
<tr>
<td>ARN-x SHR</td>
<td>15.9±1.0</td>
<td>32.6±1.4</td>
<td>16.0±1.2</td>
<td>3.20±0.24</td>
<td>2.44±0.32</td>
</tr>
</tbody>
</table>

Twenty-four hour food and water intake, urinary volume (Uv), urinary sodium (Unore+), and urinary potassium (Uk+) excretion in sham-operated and renal deafferented (ARN-x) spontaneously hypertensive rats (SHR) 7 weeks after surgery. No statistical differences in any measurement were detected (unpaired t tests). Values are mean±SEM from three collection periods.

### Table 2. Baseline Hemodynamic Measurements in Sham-operated and Renal Deafferented Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>Plasma NE (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHRyoung</td>
<td>173±6</td>
<td>361±13</td>
<td>251±31</td>
</tr>
<tr>
<td>ARN-x</td>
<td>173±8</td>
<td>344±9</td>
<td>211±34</td>
</tr>
<tr>
<td>SHRAult</td>
<td>182±3</td>
<td>330±5</td>
<td>373±56</td>
</tr>
<tr>
<td>ARN-x</td>
<td>172±3</td>
<td>321±6</td>
<td>345±38</td>
</tr>
<tr>
<td>WKY</td>
<td>125±1</td>
<td>346±8</td>
<td>ND</td>
</tr>
<tr>
<td>ARN-x</td>
<td>124±2</td>
<td>338±9</td>
<td>ND</td>
</tr>
</tbody>
</table>

Baseline mean arterial pressure (MAP), heart rate (HR), and plasma norepinephrine (NE) concentrations in renal deafferented and sham-operated spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) control rats that underwent renal deafferention (ARN-x) or sham denervation at 4 weeks (SHRyoung) or 18 weeks of age (SHRAult, WKY). ND, not determined.

*Indicates statistical difference (p<0.05) between sham-operated and ARN-x SHR (unpaired t test).
operated control rats and ARN-x SHR_{young}, mean arterial pressure was identical during the direct measurements. In ARN-x SHR_{adult}, mean arterial pressure was slightly decreased. ARN-x had no effect in WKY_{adult} rats. Heart rate was not affected by ARN-x in either group. Plasma levels of norepinephrine were not different between ARN-x rats and their sham-operated control rats in any group.

Verification of Renal Deafferentation

Renal deafferentation had no effect on renal norepinephrine content. In ARN-x SHR_{adult} and sham-operated SHR_{adult} (n=7), renal concentrations were 132±22 and 106±13 ng/g kidney wt, respectively. This indicates that renal efferent innervation was not affected by ARN-x. Bradykinin was infused intrarenally to evoke ARN-dependent increases in mean arterial pressure and heart rate. In all sham-operated rats, bradykinin infused intrarenally at a rate of 0.3 and 1.0 μg/min increased mean arterial pressure significantly (p<0.001) compared with levels reached during intravenous infusions of the same amount (see Figure 1). In ARN-x rats, the same dose of bradykinin failed to increase mean arterial pressure significantly more during intrarenal than during intravenous infusions. Interestingly, comparison of the pressor responses in sham-operated rats revealed that intrarenal as well as intravenous bradykinin infusions led to significantly (p<0.001) more potent pressor responses in intact SHR than in intact WKY rats.

Determination of Baroreceptor Reflex Sensitivity of Heart Rate

Sympathetic and vagal baroreceptor reflex control of heart rate were assessed by injections of sodium nitroprusside and phenylephrine, respectively. The results are summarized in Figure 2. Baroreceptor reflex sensitivity was higher in ARN-x SHR_{young} than in sham-operated control rats. The difference was statistically significant (p<0.05) both for phenylephrine-induced (0.68±0.09 vs. 0.40±0.07 msec/mm Hg) and sodium nitroprusside–induced (1.19±0.11 vs. 0.66±0.07 msec/mm Hg) baroreceptor reflex responses in heart rate. In SHR_{adult} renal deafferentation caused a significant (p<0.05) increase in the phenylephrine-induced baroreceptor reflex effect (ARN-x rats 0.52±13 vs. sham-operated rats 0.27±0.03 msec/mm Hg). ARN-x did not affect baroreceptor reflex sensitivity in WKY_{adult} rats.

Responses to Ganglionic Blockade

Mean arterial pressure responses to ganglionic blockade are shown in Figure 3. Intravenous bolus injection of hexamethonium decreased mean arterial pressure significantly (p<0.05) more in sham-operated SHR_{young} (57±5 mm Hg) than in ARN-x SHR_{young} (35±6 mm Hg). ARN-x had no effect on changes in mean arterial pressure induced by hexamethonium in SHR_{adult} and WKY_{adult} rats.

Discussion

The purpose of this study was to examine whether ARN contribute to the development and maintenance of an exaggerated sympathetic nervous system activity and reduced baroreceptor reflex sensitivity, which are known to develop in parallel with hypertension in SHR. Complete renal denervation techniques by surgical stripping of the renal blood vessels cannot discriminate between the afferent and efferent nerve population. We therefore performed a selective transection of the ARN by
cutting at least the dorsal roots T9-T13, which contain approximately 90% of the right renal afferent projections according to anatomic data. This procedure results in a functional deafferentation as verified by the attenuation of ARN-evoked reflex effects elicited by intrarenal bradykinin infusion in conscious rats. ARN-x does not affect the efferent renal innervation, since renal norepinephrine content was not different in sham-operated and deafferented SHR.

ARN-x, performed in prehypertensive SHR young 4 weeks after birth and in SHR adult with established hypertension, had no important influence on resting blood pressure and heart rate. Mean arterial pressure was identical in the sham-operated and ARN-x SHR young and slightly (5%) but significantly reduced in ARN-x SHR adult compared with sham-operated SHR adult. This hypotensive effect of ARN-x is small in comparison with the 20-27% reductions in arterial blood pressure after complete renal denervation in adult SHR. In other reports, however, renal denervation failed to induce a hypotensive effect in SHR when hypertension was established. Nevertheless, our data suggest that the reported hypotensive effects of total renal denervation in young as well as in adult SHR (see References 6-9,13,14,33) are mainly due to the loss of the ERN. It should be noted that the experiments were performed in the absence of atropine so that a possible muscarinic contribution to the effects cannot be excluded.

The contribution of the sympathetic nervous system to arterial blood pressure as determined by ganglionic blockade with hexamethonium did not change in SHR and WKY rats when ARN-x was performed in the adult rats. However, renal deafferentation in prehypertensive SHR young reduced the blood pressure responses to ganglionic blockade significantly compared with values reached in sham-operated SHR young. Thus ARN may contribute to the development of sympathetic nervous system activity, but are not involved in the maintenance of this system when hypertension is established in these rats. The latter part of this conclusion is in accordance with a study from Kline et al who reported that renal denervation in adult SHR did not alter central or peripheral noradrenergic activity.

Krueger et al reported that in adult SHR after complete renal denervation, blood pressure was significantly less dependent on central sympathetic tone. Comparison of our results with these observations suggests that this effect of complete renal denervation is once more mediated through the loss of the ERN. Thus ARN may contribute to the development of sympathetic nervous system activity, but are not involved in the maintenance of this system when hypertension is established in these rats. The latter part of this conclusion is in accordance with a study from Kline et al who reported that renal denervation in adult SHR did not alter central or peripheral noradrenergic activity.

The mechanisms through which a loss of the ARN population causes the aforementioned effects
are not fully understood. The results from the present studies provide evidence for the fact that in addition to central and spinal structures, peripheral sensory mechanisms in SHR may also contribute to central sympathetic tone. The hyperreactivity of SHR to sensory (stressful) stimuli and the fact that sensory deprivation and treatment with capsaicin have hypertensive effects in SHR can be interpreted as evidence for the involvement of hyperexcitable peripheral mechanisms. Furthermore the exaggerated sympathetic responses of SHR in comparison with WKY rats, when bradykinin is infused intrareally, intravenously, or injected into the carotid artery, can also be explained by an increased sensitivity of sensory mechanisms in these rats. In electrophysiological studies ARN of SHR are known to be hyperreactive compared with normotensive control rats when an ischemic stimulus is applied to the kidney. This characteristic response to hypoxia is likely not restricted to the renal afferents only. It has been reported that arterial chemoreceptor drive is augmented in SHR and also in patients with mild hypertension, leading to increased ventilatory and hemodynamic pressor responses or to exaggerated sympathetic nerve responses when acute hypoxia was induced. Taking these data together, we suggest that hypersensitivity of several sensory mechanisms may contribute to the early changes in sympathetic and cardiovascular variables in SHR.

Sympathetic and vagal baroreceptor reflex control of heart rate but not of splanchnic sympathetic nerve activity is reduced in SHR. The reduced sensitivity of baroreceptor reflex control of heart rate is due to an impaired development of a normal baroreceptor reflex sensitivity, and most likely associated with the increase in sympathetic tone and structural changes in aorta morphology. In the present experiment, the uninephrectomized SHR and WKY rats exhibited a baroreceptor reflex sensitivity comparable with that reported for two-kidney rats from our own or from other colonies. The results indicate that the vagal reflex arch, which according to Head and Adams has a reduced capacity to control heart rate in SHR, is sensitized after ARN-x in SHR and SHR. We propose that this sensitization of baroreceptor reflex control of heart rate is due to the loss of a tonic inhibition of ARN input to central structures that convey baroreceptor reflex information and to which ARN are known to project.

In ARN-x SHR but not in the ARN-x SHR, the sympathetic reflex arch also increased in sensitivity, even to values comparable with those in WKY rats. Since in these rats sympathetic tone is set to a lower level, it is likely that an adequate response of heart rate occurs to counteract the induced hypotension. In adult SHR in which the sympathetic nervous system activity has not decreased after ARN-x, heart rate responses to hypotension are impaired since sympathetic influence is already at a higher level.

In conclusion, ARN are not essential in the development and maintenance of hypertension in SHR but do affect the development of central sympathetic function and baroreceptor reflex sensitivity of heart rate in SHR. This suggests the existence of an imbalance of sympathetic-excitatory sensory mechanisms and sympatho-inhibitory baroreceptor reflex mechanisms in SHR. The causal link between these mechanisms remains to be determined.

References


**Key Words** • afferent renal nerves • baroreceptor reflexes • sympathetic nervous system • spontaneously hypertensive rats
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