Role of Afferent Renal Nerves in Spontaneous Hypertension in Rats

Ben J.A. Janssen, Helma van Essen, Lily H.T.M. Vervoort-Peters, Harry A.J. Struyker-Boudier, and Jos F.M. Smits

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-
Hypertensive Rats

Experiment 1: Effects of Renal Deafferentation in Prehypertensive Spontaneously Hypertensive Rats and Wistar-Kyoto Rats

Young female SHR (SHR_{young}) 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.\(^2\) 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 contralateral kidney.\(^4\) 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-Biosystems, 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 spectrophotometry. When blood pressure had reached a constant 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.\(^2\) The catheter was attached to an osmotic minipump (Alzet type 2001, Alza Corporation, 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 subcutaneously to the back of the neck, anchored, exteriorized, and filled with 0.9% NaCl. The osmotic minipump 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 determine the effect of ARN-x, both SHR and WKY rats were divided into two groups with comparable blood pressures, as measured with the tail-cuff method before surgery. The groups of SHR and WKY rats were subjected to ARN-x or sham surgery as described above. All rats were allowed to recover for 7-10 days. After chronic implantation of catheters (see above) the same experimental protocol was performed in all rats.

Experimental Protocol

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

Verification of renal deafferentation (day 1). Previous 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.\(^1\) 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 intervals. 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 determined as described by Smits et al.\(^2\) 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-
ephramine (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 50-0 and 0-60 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 (Coulchern 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 from three collection periods.

**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 (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.

### 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>Uno+ (meq)</th>
<th>Uko+ (meq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated SHR (n=9)</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 (n=7)</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 (Uno+), and urinary potassium (Uko+) 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>WKYyoung</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 deafferentation (ARN-x) or sham denervation at 4 weeks (SHRyoung) or 18 weeks of age (SHRyoung, 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\textsubscript{young}, mean arterial pressure was identical during the direct measurements. In ARN-x SHR\textsubscript{adult}, mean arterial pressure was slightly decreased. ARN-x had no effect in WKY\textsubscript{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\textsubscript{adult} and sham-operated SHR\textsubscript{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 \(\mu\text{g/min}\) 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\textsubscript{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\textsubscript{adult} renal deafferentation caused a significant \((p<0.05)\) increase in the phenylephrine-induced baroreceptor reflex effect \((ARN-x\text{ rats} 0.52±13\) vs. sham-operated rats 0.27±0.03 msec/mm Hg). ARN-x did not affect baroreceptor reflex sensitivity in WKY\textsubscript{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\textsubscript{young} \((57±5\) mm Hg) than in ARN-x SHR\textsubscript{young} \((35±6\) mm Hg). ARN-x had no effect on changes in mean arterial pressure induced by hexamethonium in SHR\textsubscript{adult} and WKY\textsubscript{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
Figure 2. Bar graphs showing baroreceptor reflex sensitivity (BRS) to phenylephrine and nitroprusside injections in renal deafferented (ARN-x) or sham-operated (SHAM) rats at 4 weeks (young) and 22 weeks (adult) of age. *Statistical difference \( p<0.05 \) between sham-operated and ARN-x rats. °Statistical difference \( p<0.001 \) between Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR).

Figure 3. Bar graph showing effects of ganglionic blockade (hexamethonium) on mean arterial pressure (MAP) in renal deafferented (ARN-x) or sham-operated (SHAM) rats at 4 weeks (young) and 22 weeks (adult) of age. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. * Statistical difference \( (p<0.05) \) between sham-operated and ARN-x rats.

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.

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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 hypotensive 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 intrarenally, 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 hyperreactivity 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, the sympathetic reflex arc 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 sympatho-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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