Autonomic control of the heart is altered in Sprague-Dawley rats with spontaneous hydronephrosis

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Arnold AC, Shaltout HA, Gilliam-Davis S, Kock ND, Diz DI. Autonomic control of the heart is altered in Sprague-Dawley rats with spontaneous hydronephrosis. Am J Physiol Heart Circ Physiol 300:H2206–H2213, 2011. First published April 1, 2011; doi:10.1152/ajpheart.01263.2010.—The renal medulla plays an important role in cardiovascular regulation, through interactions with the autonomic nervous system. Hydronephrosis is characterized by substantial loss of renal medullary tissue. However, whether alterations in autonomic control of the heart are observed in this condition is unknown. Thus we assessed resting hemodynamics and baroreflex sensitivity (BRS) for control of heart rate in urethane/chloralose-anesthetized Sprague-Dawley rats with normal or hydronephrotic kidneys. While resting arterial pressure was similar, heart rate was higher in rats with hydronephrosis (290 ± 12 normal vs. 344 ± 11 mild/moderate vs. 355 ± 13 beats/min severe; P < 0.05). The evoked BRS to increases, but not decreases, in pressure was lower in hydronephrotic rats (1.06 ± 0.06 normal vs. 0.72 ± 0.10 mild/moderate vs. 0.63 ± 0.07 ms/mmHg severe; P < 0.05). Spectral analysis methods confirmed reduced parasympathetic function in hydronephrosis, with no differences in measures of indirect sympathetic activity among conditions. As a secondary aim, we investigated whether autonomic dysfunction in hydronephrosis is associated with activation of the renin-angiotensin system (RAS). There were no differences in circulating angiotensin peptides among conditions, suggesting that the impaired autonomic function in hydronephrosis is independent of peripheral RAS activation. A possible site for angiotensin II-mediated BRS impairment is the solitary tract nucleus (NTS). In normal and mild/moderate hydronephrotic rats, NTS administration of the angiotensin II type I receptor antagonist candesartan significantly improved the BRS, suggesting that angiotensin II provides tonic suppression to the baroreflex. In contrast, angiotensin II blockade produced no significant effect in severe hydronephrosis, indicating that at least within the NTS baroreflex suppression in these animals is independent of angiotensin II.

baroreflex; solitary tract nucleus; angiotensin; spectral analysis

EMERGING EVIDENCE IMPLICATES reciprocal interactions between the brain and kidneys for cardiovascular regulation, in part mediated by the autonomic nervous system (9, 21). The progression of numerous renal diseases is associated with impairments in autonomic regulation of the heart, including baroreflex dysfunction and reductions in heart rate variability. The loss of ability to effectively regulate blood pressure in these conditions may be in part due to altered function of the renal medulla, which is directly innervated by the autonomic nervous system and exerts antihypertensive actions through a variety of mechanisms (6). Deterioration of the renal medulla is observed in hydronephrosis, a condition that can occur in response to ureteral obstruction or spontaneously, independent of obstruction in humans and animal models (14, 38, 39, 42, 43). Regardless of the pathogenesis, hydronephrosis is characterized by progressive dilatation of the renal pelvis, resulting in urine accumulation and substantial loss of renal medullary tissue (22). Whether cardiovascular regulation is altered in hydronephrosis has yet to be investigated. Thus, the present study employed established pharmacological and spectral analysis tools to assess autonomic control of the heart in Sprague-Dawley rats, a strain with a historically frequent occurrence of spontaneous hydronephrosis (5, 46).

One hormonal system intimately involved in autonomic and renal regulation is the renin-angiotensin system (RAS). Activation of the RAS has been shown to play an important role in the pathophysiology of many renal diseases, including renovascular hypertension, acute renal failure, and obstructive nephropathy (17, 21). The main effector peptide of the RAS, angiotensin II, acts at autonomic nuclei within the central nervous system to alter autonomic control of heart rate (27). Elevations in angiotensin II, either peripherally or centrally, increase sympathetic nervous system activity to organs involved in cardiovascular regulation and decrease heart rate variability and baroreflex sensitivity (BRS) for control of heart rate, important indexes of parasympathetic outflow to the heart, at sites accessed by the systemic circulation as well as within the blood-brain barrier (7, 26, 27, 44). As a secondary aim, we evaluated whether the autonomic dysfunction in animals with spontaneous hydronephrosis is associated with activation of the circulating RAS. We further evaluated whether impairments in baroreflex function in hydronephrosis involve a central angiotensin II component within the solitary tract nucleus (NTS), an autonomic brain stem region mediating baroreflex function (2).

METHODS

The following procedures were approved by the institutional animal care and use committee and have been reported in detail in recent publications (3, 4).

Animals. Experiments were performed in 3- to 5-mo-old male Hannover Sprague-Dawley rats obtained from the Hypertension and Vascular Research Center Colony at the Wake Forest University School of Medicine in Winston-Salem, NC. While spontaneous hydronephrosis is consistently present in our colony, we have observed that the incidence can widely fluctuate depending on time of year, similar to a previous report (29). At the time of these studies, ~70% of our animals exhibited hydronephrosis, an incidence within the range of historically reported values for normotensive rat strains (5, 29, 46).

Circulating angiotensin peptides. Naive conscious or urethane/chloralose-anesthetized Sprague-Dawley rats were killed by rapid decapitation, and trunk blood (~8 ml) was collected in the presence of protease inhibitors for radioimmunoassay measurement of plasma...
angiotensin I, angiotensin II, and angiotensin (1–7) as previously described (18).

**Surgical procedures.** In a separate group of animals, combination urethane/chloralose anesthesia (750 mg and 35 mg/kg, respectively) was administered via intraperitoneal injections, with intravenous supplemental doses given as needed. Catheters were inserted in the femoral arterial and vein for cardiovascular parameter measurement and drug delivery, respectively. All animals were placed in a stereotaxic frame with the head tilted downward (45°) for surgical exposure of the dorsal medulla oblongata. After surgical procedures, a 30-min period was allowed before taking resting hemodynamic measurements.

**Resting hemodynamic measures.** Resting hemodynamics, BRS, and spectral analysis indexes were obtained in rats with normal (n = 7), mild/moderate (n = 11), and severe (n = 11) hydronephrosis. A strain gauge transducer connected to the femoral artery was used to monitor, record, and digitize pulsatile arterial pressure and mean arterial pressure (MAP) using a Data Acquisition System (Acknowledgement software version 3.8.1; BIOPAC System) with heart rate determined from the arterial pressure wave.

**Reflex testing.** The BRS in response to increases or decreases in arterial pressure was determined by bolus randomized intravenous administration of phenylephrine or sodium nitroprusside (2, 5, and 10 µg/kg in 0.9% NaCl), respectively. Because angiotensin peptides selectively alter the BRS to increases in arterial pressure (7, 33), we studied transient responses to bolus injections, which are more sensitive to parasympathetic alterations relative to ramp responses with infusions (23). Maximum MAP responses (ΔMAP, mmHg) and associated reflex changes in heart rate (ΔHR, beats/min) were recorded at each dose of phenylephrine or nitroprusside, and ΔHR was converted to changes in pulse interval (ΔPI, ms) by the formula: 60,000/HR. The slope of the line fit through the ΔMAP and corresponding ΔPI was used as an index of BRS for control of heart rate.

**Spectral analysis.** As previously reported (4, 40), spontaneous BRS and other indexes of sympathetic-vagal balance were assessed by post hoc spectral analysis of arterial pressure and heart rate recordings (N evroka rd SA-BRS software; Medistar, Ljubljana, Slovenia). Consistent with the duration of recordings in previous rodent and human studies (4, 13, 30, 40), the spontaneous BRS was determined from a minimum of 5 min of recordings taken before the evoked baroreflex testing. To calculate the spontaneous BRS, power spectral densities of systolic arterial pressure (SAP) and beat-to-beat interval (RRI) oscillations were computed, transformed, and integrated over specified frequency ranges [low frequency (LF) = 0.25–0.75 Hz; high frequency (HF) = 0.75–3.0 Hz]. The square root of the ratio of RRI and SAP powers was used to calculate HF/α and LF/α components, which reflect parasympathetic and primarily sympathetic activity of the spontaneous BRS, respectively. The power of RRI spectra in the LF and HF range (LF_	ext{RRI} and HF_	ext{RRI}) was calculated, and the ratio of LF_	ext{RRI} to HF_	ext{RRI} was used as an index of cardiac sympathovagal balance, similar to previous reports (1, 31). The LF component of the SAP variability (LF_	ext{SAP}) was calculated in normalized units (nu) and was used as an indirect measure of sympathetic activity. Heart rate variability was measured in the time domain as the standard deviation of the RRI as well as the coefficient of variance to account for differences in resting heart rate among conditions. Blood pressure variability was measured as the standard deviation of the MAP by time domain analysis methods.

**NTS candesartan microinjection.** In a subset of animals (n = 4 each group), we performed bilateral NTS microinjection of the angiotensin II type 1 (AT₁) receptor antagonist candesartan at a dose found functionally effective in previous studies [CV-11974; 24 pmol/120 nl dissolved in artificial cerebrospinal fluid; pH 7.4; Takeda Chemical Industries (7, 26)]. At least 30 min were allowed after baseline reflex testing before commencing microinjections. Multibarreled glass pipettes were used to bilaterally inject candesartan via pressure in the NTS [0.4 mm rostral, 0.4 mm lateral to the calamus scriptorius (caudal tip of the area postrema), and 0.4 mm below the dorsal surface]. BRS testing was repeated at 10 min after candesartan injection so that each animal was used as its own control, and all reflex testing was completed within 20 min. Previous studies in our laboratory show that vehicle injection of artificial cerebrospinal fluid (120 nl) has no effect on arterial pressure, heart rate, or BRS in urethane/chloralose-anesthetized rats (4, 35). In rats receiving candesartan injections, the brain was removed and frozen on dry ice for histological evaluation. Serial cryostat sections (30 µm) of the frozen medulla were used to assess the site of microinjections. Only experiments in which the pipette tip was localized within the medial NTS at rostrocaudal level −13.3 to −14.0 mm caudal to bregma were included in this study with a success rate ≥90%, similar to previous reports (3, 4).

**Kidney histology.** At the end of experiments, rats were killed by rapid decapitation. Kidneys were removed, cut along the longitudinal axis, and visually classified as normal or hydronephrotic. The degree of hydronephrosis was defined as mild, moderate, or severe based on established criteria examining the extent of renal medullary damage (48). Kidneys were classified as I normal, if there was no or minimal space between the papilla and calyx; 2) mild, if there was a narrow but definable fluid-filled calyceal space with normal papillary shape; 3) moderate, if there was dilation of the calyx with compression of the papilla but preserved shape; and 4) severe, if there was gross distension of the calyx, severe compression of the lateral cortex, and distortion of the papilla. Only animals exhibiting bilateral hydronephrosis were included, and only rats with two unaffected kidneys were considered normal. Kidneys were fixed in 10% formalin and embedded in paraffin, and serial cuts (5 µm) were stained with hematoxylin and eosin for morphological analysis by standard techniques.

**Markers of renal function.** Similar to previous reports in the literature (14, 22, 43), we assessed for markers of renal function in a separate group of conscious animals with normal or hydronephrotic kidneys using previously established methods (15). To maintain consistency with previous reports, we did not separate these animals by degree of hydronephrosis. Systolic blood pressure was measured using the tail-cuff method. Animals were acclimated to the device by training sessions, and the mean of at least five blood pressure measurements was determined for each animal. Animals were weighed and placed in metabolic cages for 24-h urine collection on dry ice.

![Fig. 1. Histological analysis of kidneys.](http://ajpheart.physiology.org/)

**Fig. 1.** Histological analysis of kidneys. Histological analysis of representative hematoxylin- and eosin-stained kidney sections (5 µm). Kidneys were classified as normal (A) or hydronephrotic, with the degree of hydronephrosis being mild/moderate (B) or severe (C) based on the extent of renal medullary damage.
Table 1. Markers of renal function in conscious Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (n = 3)</th>
<th>Hydronephrotic (n = 5)</th>
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<tbody>
<tr>
<td>SBP, mmHg</td>
<td>134 ± 1</td>
<td>134 ± 2</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>493 ± 21</td>
<td>520 ± 18</td>
</tr>
<tr>
<td>Urine volume, ml/day</td>
<td>9 ± 1</td>
<td>14 ± 1*</td>
</tr>
<tr>
<td>Urine creatinine, mg · kg⁻¹ · day⁻¹</td>
<td>28 ± 2</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Serum creatinine, mg/dl</td>
<td>0.42 ± 0.08</td>
<td>0.58 ± 0.03</td>
</tr>
<tr>
<td>Proteinuria, mg/day</td>
<td>13 ± 3</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>Urine angiotensin I, pmol · mg⁻¹ · day⁻¹</td>
<td>0.13 ± 0.04</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Urine angiotensin II, pmol · mg⁻¹ · day⁻¹</td>
<td>0.27 ± 0.04</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>Urine angiotensin-(1–7), pmol · mg⁻¹ · day⁻¹</td>
<td>0.12 ± 0.03</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Data represent systolic blood pressure (SBP), body weight, and markers of renal function in conscious Sprague-Dawley rats with normal or hydronephrotic kidneys. *P < 0.05 vs. normal.

Urine volume was significantly higher in rats with hydronephrosis relative to normal rats (Table 1). The increases in tail cuff systolic blood pressure, body weight, proteinuria, urinary angiotensin peptides, and urine or serum creatinine were also not significantly different among conscious animals with normal or hydronephrotic kidneys (Table 2).

**RESULTS**

Kidney histology. The degree of hydronephrosis was defined as mild, moderate, or severe based on the extent of renal medullary damage (Fig. 1). Severe hydronephrosis was accompanied by accumulation of urine in the renal pelvis. Aside from deterioration of the renal medulla, there was no other gross pathology evident in hydronephrotic kidneys. Histological examination of hematoxylin- and eosin-stained kidneys revealed no significant changes, other than hydronephrosis.

Markers of renal function. In a subset of conscious rats with normal or hydronephrotic kidneys, there were no significant differences in tail cuff systolic blood pressure, body weight, proteinuria, urinary angiotensin peptides, and urine or serum creatinine (Table 1). Urine volume was significantly higher in rats with hydronephrotic relative to normal kidneys (P < 0.05, Table 1).

Circulating angiotensin peptides. Data from anesthetized animals with mild and moderate hydronephrosis were pooled since there were no significant differences in any of the circulating angiotensin peptides or resting hemodynamic or reflex measurements between these groups. There were no significant differences in circulating levels of angiotensin I, angiotensin II, or angiotensin-(1–7) among anesthetized rats with normal, mild/moderate, or severe hydronephrotic kidneys (Fig. 2). Plasma levels of angiotensin peptides were not significantly different among conscious animals with normal or hydronephrotic kidneys (Table 2).

Resting hemodynamics and baroreflex function in anesthetized rats. While resting arterial pressure was similar among all conditions, heart rate was significantly higher in mild/moderate and severe hydronephrotic relative to normal rats (P < 0.05, Table 3). The BRS for bradycardia to increases in arterial pressure evoked by phenylephrine was significantly lower in mild/moderate and severe hydronephrotic relative to normal rats (P < 0.05, Fig. 3, B and C). There was no significant difference in the level of BRS impairment between rats with mild/moderate and severe hydronephrosis. The increases in MAP induced by the lower and middle doses of phenylephrine

![Fig. 2. Circulating levels of angiotensin peptides in anesthetized Sprague-Dawley rats.](http://ajpheart.physiology.org/)
Table 2. Circulating angiotensin peptides in conscious Sprague-Dawley rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Angiotensin I, pg/ml</th>
<th>Angiotensin II, pg/ml</th>
<th>Angiotensin-(1-7), pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>9</td>
<td>101 ± 13</td>
<td>27 ± 4</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>9</td>
<td>84 ± 8</td>
<td>30 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>Severe</td>
<td>9</td>
<td>110 ± 31</td>
<td>22 ± 8</td>
<td>20 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Data represent circulating angiotensin peptides in conscious Sprague-Dawley rats with normal, mild/moderate, or severe hydropnephrotic kidneys.

were similar among conditions (Fig. 3A). However, increases in pressure produced by the highest dose of phenylephrine were significantly higher in animals with severe hydropnephrosis relative to normal rats (P < 0.05). The BRS for tachycardia to decreases in arterial pressure evoked by nitroprusside was similar among rats with normal and hydropnephrotic renal kidneys (Fig. 3D).

Indexes of sympathovagal balance in anesthetized rats. There were no significant differences in baseline spectral analysis measures between rats with mild/moderate and severe hydropnephrosis. The HF \( \text{pectral} \) component of the spontaneous BRS was lower in rats with mild/moderate and severe hydropnephrosis compared with normal rats (P < 0.05, Fig. 4A). The LF \( \text{rhythmic} \) component of the peripheral autonomic nervous system was higher in rats with severe hydropnephrosis (P < 0.05, Fig. 4B). As a result, the LF \( \text{rrt} \)-to-HF \( \text{rrr} \) ratio was significantly higher in rats with severe hydropnephrosis (P < 0.05, Fig. 4C), with a similar trend for mild and moderate severe hydropnephrosis. Resting heart rate variability was significantly lower in rats with mild/moderate and severe hydropnephrosis (P < 0.01, Fig. 4, D and E) relative to normal rats. There were no significant differences in blood pressure variability (2.1 ± 0.2 normal vs. 2.5 ± 0.4 mild/moderate vs. 2.3 ± 0.2 mmHg severe) or LF \( \text{spat} \) (15.3 ± 2.2 normal vs. 12.9 ± 2.2 mild/moderate vs. 14.6 ± 1.8 severe) among conditions.

Effect of NTS candesartan injection on the BRS. Baseline values of the BRS for animals used in the microinjection studies did not differ significantly from the larger group data used to assess autonomic regulation. In anesthetized rats with normal or mild/moderate hydropnephrotic kidneys, candesartan injection significantly facilitated the evoked BRS for bradycardia by ~ 32 and 46%, respectively (Fig. 5). After candesartan injection, the BRS was not significantly different between normal and mild/moderate hydropnephrosis animals. Candesartan injection produced no significant effect on the bradycardic BRS in animals with severe hydropnephrosis (Fig. 5).

DISCUSSION

The primary goal of the present study was to determine whether hydropnephrosis, a condition characterized by loss of renal medullary tissue, is associated with alterations in autonomic control of the heart. Our findings show that Sprague-Dawley rats with spontaneous hydropnephrosis exhibit impairments in autonomic regulation, including 1) higher resting heart rate, with no differences in arterial pressure; 2) impairments in indexes of parasympathetic function, including heart rate variability and the BRS for bradycardia; 3) a shift in sympathovagal balance toward sympathetic dominance; and 4) no differences in indirect indexes of sympathetic activity. As a secondary goal, we investigated whether activation of the RAS was associated with the autonomic dysfunction in these animals. These studies provide evidence that circulating angiotensin peptides are not significantly altered in conscious or anesthetized animals with spontaneous hydropnephrosis, suggesting that the autonomic changes are not associated with activation of the peripheral RAS. Independent of the circulating RAS, brain angiotensin II is implicated in altering auto-

Fig. 3. a-Adrenergic responsiveness and resting baroreflex function. A: changes in mean arterial pressure (MAP) in response to iv phenylephrine boluses are shown for rats with normal, mild/moderate, and severe hydropnephrosis (n = 7, 11, and 11, respectively). There were no significant differences in phenylephrine-induced pressor responses between normal rats and animals with mild/moderate hydropnephrosis. Although the low and middle doses were similar, pressor responses to the highest dose of phenylephrine were significantly higher in rats with severe hydropnephrosis relative to normal animals. B: the baroreflex sensitivity (BRS) for bradycardia to increases in arterial pressure evoked by phenylephrine was significantly lower in rats with mild/moderate and severe hydropnephrosis relative to rats with normal kidneys. C: the slope of the relationship between the increases in MAP produced by phenylephrine and the corresponding reflex bradycardia [expressed as the pulse interval (PI)] shows a reduction in the linear regression slope in mild/moderate and severe hydropnephrosis relative to animals with normal kidneys (0.98 ± 0.10 normal vs. 0.75 ± 0.11 mild/moderate vs. 0.55 ± 0.08 ms/mmHg severe (r² = 0.60–0.80)]. D: the BRS for tachycardia to decreases in arterial pressure evoked by nitroprusside was not different among groups. *P < 0.05 vs. normal.
nomic balance at central sites of action (27). Thus, we examined whether the BRS impairment in hydronephrosis involves a central angiotensin II component within the NTS, a key brainstem region mediating baroreflex function (2). Microinjection of the angiotensin II AT₁ receptor antagonist candesartan within the NTS improved the BRS in mild/moderate hydronephrosis to a similar level as normal rats, suggesting that increased angiotensin II actions within the NTS may contribute to the baroreflex dysfunction in these animals. In contrast, there was no significant effect of candesartan injection in rats with severe hydronephrotic kidneys, suggesting angiotensin II-independent mechanisms for baroreflex suppression in severe hydronephrosis.

While ureteral obstruction is a common cause of hydronephrosis in humans, this condition also occurs spontaneously, without obstruction in humans and rodents (14, 38, 39, 42, 43). At the present time, we do not know the cause of hydronephrosis in Sprague-Dawley rats; however, previous reports describe genetic inheritance patterns for spontaneous hydronephrosis in other rat strains (22, 43). Previous reports suggest that hydronephrosis can be associated with mild impairments in renal excretory function and glomerular filtration rate and consistent severe impairments in renal concentrating ability (22, 42, 43), resulting in increased urine volume. Similar to previous reports (14, 38, 39), histological analysis of kidneys from rats with hydronephrosis revealed atrophy of the renal medulla with no alterations in the morphology of the remaining renal tissue. Because we observed marked atrophy of the renal medulla, we focused on analysis of urine volume, a marker directly influenced by this part of the kidney, in a subset of normal and hydronephrotic animals. Indeed, polyuria was observed in rats with hydronephrosis, indicating impaired renal concentrating capacity and consistent with previous reports (22, 43). To characterize our colony in the context of previous literature findings, we also analyzed for other markers of renal function. Consistent with a lack of alteration in renal cortical and glomerular structures upon histological examination, there were no significant changes in proteinuria, a marker of renal damage, or in urine and serum creatinine, suggesting intact glomerular filtration in these animals. Urinary angiotensin peptide excretion was also similar among conditions, suggesting that our model of hydronephrosis is not associated with activation of the intrarenal RAS.

Similar to previous reports in humans and rodents (22, 42, 43), resting blood pressure was not altered in anesthetized or conscious rats with spontaneous hydronephrosis. The normotensive status in the face of impaired BRS suggests a pressure-independent mechanism for baroreflex dysfunction in hydronephrotic rats. These findings are consistent with previous studies showing that the BRS is controlled independently from the set point of the baroreflex (3, 4, 35). While not examined in the present study, rats with spontaneous hydronephrosis are...
reported to exhibit greater sensitivity to a mild hypertensive salt stimulus (41) and have a slightly higher incidence of hypertension (43) relative to normal rats. These findings may be due to impairments in renomedullary antihypertensive mechanisms as well as the alterations in autonomic regulation described in the present study. Because the heart is under tonic inhibitory control by the parasympathetic nervous system, the elevation in resting heart rate in Sprague-Dawley rats with hydrenchrosis suggests reduced parasympathetic function. Importantly, elevations in resting heart rate are positively associated with an increase in all-cause mortality, independent of elevations in arterial pressure (45).

The use of pharmacological agents is an established tool to evaluate baroreceptor reflex control of sympathetic and parasympathetic activities to the heart. The BRS for bradycardia evoked by phenylephrine, an established measure of vagal function (32, 45), was reduced in hydrenchrotic rats to similar levels as hypertensive animals (11). The lower BRS in animals with mild/moderate hydrenchrosis was not attributed to differences in α-adrenergic responsiveness, since pressor responses to phenylephrine were similar relative to normal animals. However, we observed increased adrenergic responsiveness at the highest dose of phenylephrine in animals with severe hydrenchrosis. Despite a higher blood pressure response, rats with severe hydrenchrosis exhibited lower heart rate responses, indicating a decrease in reflex vagal restraint. The finding of increased vasoconstrictor responsiveness to phenylephrine has also been observed in human and animal models of hypertension (24, 49). It is possible that there is a difference in the duration of increase caused by the bolus injections, related to the differences in the reduction of heart rate related to BRS, but this is not evaluated as part of the baroreflex measures. There were no differences in the BRS for tachycardia evoked by nitroprusside among conditions, suggesting intact baroreflex control of sympathetic activity to the heart. The resting tachycardic BRS, although lower than the bradycardic BRS, is within the range of previously reported values for urethane/chloralose-anesthetized rats using the same methods (4, 7). The selective reduction in the parasympathetic, but not sympathetic, component of the BRS in hydrenchrotic rats is similar to central actions of angiotensin II (7, 33) and mimics observations in human hypertension (16).

To complement evoked pharmacological measures of BRS, we employed spectral analysis methods. Although controversial, spectral analysis is widely used to indirectly assess markers of sympathetic and parasympathetic regulation of the heart (1, 8, 20, 50). The classic pharmacological method estimates baroreflex function during large changes in arterial pressure evoked by vasoactive agents in an open-loop dynamic system. In contrast, the spontaneous method estimates the baroreflex by measuring beat-to-beat oscillations in blood pressure and heart rate over a smaller, normal physiological range in a closed-loop system. Although a highly significant correlation exists between BRS values obtained by evoked and spectral analysis methods, differences in the absolute values have been reported because of differences in the sensitivities of these methods (32, 40). Consistent with the present study, previous reports show that the BRS value measured from phenylephrine is often lower than that obtained with the spontaneous method (4, 19, 34). The HF component of the spontaneous BRS, a marker of parasympathetic activity that is abolished by atropine (8, 20, 32), was similar in anesthetized animals relative to historical data for conscious Sprague-Dawley rats (40). This finding suggests that, even under anesthesia, rats with normal kidneys maintain the ability to respond to beat-to-beat physiological oscillations. In animals with hydrenchrosis, both the evoked and spontaneous BRS were reduced, suggesting impairment in the ability to respond to changes in blood pressure under both

Table 3. Resting arterial pressure and heart rate

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Resting AP, mmHg</th>
<th>Resting HR, beats/min</th>
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<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>95 ± 5</td>
<td>290 ± 12</td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>11</td>
<td>103 ± 5</td>
<td>341 ± 10*</td>
</tr>
<tr>
<td>Severe</td>
<td>11</td>
<td>99 ± 3</td>
<td>325 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Data represent resting arterial pressure (AP) and heart rate (HR) in urethane/chloralose-anesthetized Sprague-Dawley rats with normal, mild/moderate, or severe hydrenchrotic kidneys. *P < 0.05 vs. normal.
natural and dynamic conditions. The lower BRS and heart rate variability, an indirect measure of vagal tone to the heart, in animals with hydronephrosis are suggestive of impaired parasympathetic function. The lower LF-SAP in rats with severe hydronephrosis may be in part due to the reduction in vagal tone, since the spectral density of arterial pressure in the LF range is partially controlled by vagal tone (8, 20). Thus, either a reduction in parasympathetic or increase in sympathetic tone could contribute to the shift in sympathovagal balance toward sympathetic dominance in severe hydronephrotic rats. Although we did not directly measure sympathetic outflow, either whole body or organ-specific, there were no differences in indirect measures of sympathetic activity, including LF-SAP and blood pressure variability among conditions. Importantly, LF-SAP is abolished after sympathetic blockade in humans and rodents and tracks closely with changes in directly measured peripheral nerve activity (8, 20, 31). Overall, the present findings suggest that impairments in autonomic regulation of the heart in rats with hydronephrosis are primarily associated with reduced parasympathetic tone.

The results of the present study suggest that circulating or urinary angiotensin peptides are not altered in rats with hydronephrosis, despite profound differences in autonomic control of heart rate. Thus, the autonomic dysfunction in rats with hydronephrosis does not appear to be associated with activation of the peripheral or intrarenal RAS. A previous report showed that rats with hereditary hydronephrosis exhibit hyperkalemia, which could suppress the circulating RAS (43). However, in that study, animals still had significantly higher basal levels of plasma renin activity, suggesting that potassium may not influence the systemic RAS in this condition. Independent of the circulating system, the brain RAS can also contribute to autonomic imbalance and the development of hypertension. In both conscious and anesthetized normotensive rats, microinjection of AT1 receptor antagonists improves the BRS for bradycardia by 30–40%, suggesting that angiotensin II endogenous to the NTS provides tonic suppression to baroreflex function under normal conditions (7, 10, 28). In the present study, NTS injection of candesartan significantly facilitated the BRS in rats with normal kidneys by 32%, consistent with these previous reports. Candesartan injection also improved the BRS in rats with mild/moderate hydronephrosis to a similar level as normal animals, providing evidence that increased angiotensin II actions within the NTS may contribute to the BRS impairment in these animals.

Despite a similar resting level of BRS in rats with mild/moderate and severe hydronephrosis, acute AT1 receptor blockade within the NTS did not significantly alter the BRS in rats with severe hydronephrosis. These data suggest that at least within the NTS angiotensin II-independent mechanisms contribute to the impaired BRS in severe hydronephrosis, whereas there is an angiotensin II-dependent component of the BRS in normal and mild/moderate hydronephrotic animals. These findings support that a dysregulation of the brain RAS within the NTS may occur in severe hydronephrosis. As a potential mechanism for brain RAS dysregulation, the lack of effect of candesartan on the BRS is similar to observations in transgenic hypertensive (mRen2)27 rats (10) where acute angiotensin II blockade is not enough to overcome the consequences of long-term exposure to elevated brain angiotensin II levels and subsequent changes in functional and signaling pathways in medullary and brain stem regions modulating autonomic outflow (47). A potential limitation of this study is that the anesthesia may influence cardiovascular and autonomic function in these animals. However, this study employed urethane/chloralose, a combination anesthesia widely used for animal studies investigating neural control of the circulation because of its relative preservation of autonomic function compared with other anesthetics. Under this anesthesia, the relative levels of resting baroreflex function mimic patterns observed in conscious animals (4, 25, 37). Furthermore, we and others have shown that NTS administration of AT1 receptor antagonists produces a similar percent increase in the BRS in anesthetized relative to conscious conditions (10, 28, 36), suggesting that this anesthesia does not confound the interpretation of candesartan effects on baroreflex function.

In conclusion, the present study provides evidence that spontaneous hydronephrosis in Sprague-Dawley rats is associated with impairments in autonomic control of heart rate, independent of activation of the peripheral RAS and perhaps involving increased angiotensin II actions within the NTS for baroreflex modulation in mild/moderate hydronephrosis. At the present time, we do not know the mechanisms underlying baroreflex suppression in rats with severe hydronephrosis. We cannot completely rule out a role for the brain RAS in modulation of baroreflex function in these animals, since this condition may be associated with reduced central levels of angiotensin-(1–7), a peptide that opposes actions of angiotensin II on the BRS (11), or with altered sensitivity to angiotensin peptides at other autonomic brain sites involved in regulation of sympathetic and parasympathetic outflow (12). There are numerous other possibilities that could also contribute to the BRS impairment in these animals, including local renal mechanisms, alterations in renal sympathetic nerve activity, and changes in nitric oxide, GABA, glutamate, reactive oxygen species, inflammatory molecules, and monoamine or neuromodulator transmitters (i.e., norepinephrine, serotonin, aldosterone, vasopressin, insulin, and leptin). Understanding the mechanisms of autonomic dysfunction in these animals may provide new insight into interactions between the brain and kidney for cardiovascular regulation in hydronephrosis.

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DISCLOSURES
None.

REFERENCES


