Adrenergic regulation of (Na\(^+\), K\(^+\))-ATPase activity in proximal tubules of spontaneously hypertensive rats

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Adrenergic regulation of (Na\(^+\), K\(^+\))-ATPase activity in proximal tubules of spontaneously hypertensive rats. Increased renal nerve activity and sodium retention have been implicated in the development of hypertension in genetically transmitted forms of this disease. The present studies were designed to investigate the relationship between renal nerve integrity and renal proximal tubule (Na\(^+\), K\(^+\))-ATPase activity in spontaneously hypertensive rats (SHR). (Na\(^+\), K\(^+\))-ATPase activity of basolateral membranes (BLMs) enriched from proximal tubules of five-week-old SHR was greater, 328.6 ± 18.9 nmol P/mg protein · min, than in age-matched genetic controls rats (Wystar-Kyoto, WKY, rats), 262.3 ± 34.6 nmol P/mg protein · min (P < 0.02). There was no detectable difference in (Na\(^+\), K\(^+\))-ATPase activity of 13-week-old SHR and WKY rats. Prior renal denervation was associated with a reduction in proximal tubule basolateral membrane (BLM) (Na\(^+\), K\(^+\))-ATPase activity, 316.8 ± 23.8 to 223.1 ± 23.9 nmol P/mg protein · min (P < 0.02), in five-week SHR. However, denervation had no effect on renal (Na\(^+\), K\(^+\))-ATPase activity in either WKY rats, nor did sham-denervation in SHR. In addition, exogenous norepinephrine, 1 μM, produced a more pronounced stimulation of (Na\(^+\), K\(^+\))-ATPase activity in basolateral membranes from SHR as opposed to WKY controls (40.2% vs. 28.7%). Therefore, renal nerve integrity and exogenous catecholamines have a greater stimulatory influence on proximal tubule (Na\(^+\), K\(^+\))-ATPase activity in the early stages (prior to 5 weeks) of the development of hypertension in SHR than in age-matched WKY rats. These findings suggest that increased renal nerve activity and enhancement of proximal tubule (Na\(^+\), K\(^+\))-ATPase activity by catecholamines may contribute to salt retention and the development of hypertension in the SHR model of hypertension.

It is widely accepted that the kidney is involved primarily in the pathogenesis of genetic hypertension in rats. Central to this hypothesis is the observation that spontaneously hypertensive rats (SHR), as opposed to normotensive controls, retain sodium avidly prior to the development of hypertension [1]. Furthermore, dietary sodium restriction retards the development of hypertension in SHR [2, 3]. In addition, transplantation of kidneys from genetically similar normotensive rats into SHR normalizes blood pressure, while in contrast, transplantation of kidneys from hypertensive to normotensive rats is associated with the development of hypertension [4].

Since renal nerve activity has been observed to be greater in SHR [5] and since increased efferent renal nerve activity enhances proximal tubular sodium reabsorption [6, 7], it has been suggested that renal nerve activity participates in the renal contribution to the development of genetic hypertension. Moreover, renal denervation performed prior to established hypertension delays the onset and reduces the severity of hypertension in SHR strains of rat [8–11]. However, the mechanism by which renal nerve activity may contribute to sodium retention and the development of hypertension has not been elucidated. Nevertheless, recent studies have demonstrated that norepinephrine stimulates proximal tubular (Na\(^+\), K\(^+\))-ATPase activity in rabbits [12], thus suggesting one potential mechanism whereby renal nerve activity may modulate sodium reabsorption.

The present studies were designed to examine if increased renal nerve activity or sensitivity to catecholamines is related to (Na\(^+\), K\(^+\))-ATPase activity in proximal tubules of young SHR. The results show that early in or prior to the development of overt hypertension SHR have an increased proximal tubule (Na\(^+\), K\(^+\))-ATPase activity as compared to WKY rats. Moreover, denervation was demonstrated for the first time to ameliorate this enhanced proximal tubular BLM (Na\(^+\), K\(^+\))-ATPase activity in SHR, while having no effect in WKY rats. Furthermore, stimulation of (Na\(^+\), K\(^+\))-ATPase activity by norepinephrine was greater in SHR than WKY rats. Thus, sodium retention and the development of hypertension in SHR may be secondary, in part, to enhanced renal nerve activity and increased sensitivity to neurotransmitters that augment sodium pump activity and proximal tubular sodium reabsorption.

Methods

Animal groups

Male Wistar-Kyoto (WKY) and spontaneously hypertensive-Ookamoto strain-rats (SHR) were obtained from Charles River Breeding Lab (Wilmington, Massachusetts, USA) at 24 and 80 days of age. The animals were housed in separate cages and given free access to standard rat chow (Ralston Purina, St. Louis, Missouri, USA) and tap water.

After 48 hours acclimatization, tail-cuff systolic arterial blood pressures were obtained using a pneumatic pulse transducer (IITC, Landen, New Jersey, USA) and physiograph recorder (Hewlett-Packard, Palo Alto, California, USA) daily for four days. The recordings of the first one or two days were considered as acclimatization and were not included. At least two recordings were obtained each day and averaged for each
animal. At the end of 10 days the rats were sacrificed and the kidneys removed for preparation of proximal tubule basolateral membranes.

**Basolateral membrane preparation**

In order to more directly assess the (Na\(^+\), K\(^+\))-ATPase activity of renal proximal tubules, basolateral membranes (BLMs) of proximal tubule origin were separated and enriched for measurement of sodium pump activity in the presence of maximal substrate concentrations. BLMs from rat cortex were prepared by the method of Sacktor et al [13] as modified by Schwab, Klahr and Hammerman [14]. In brief, homogenized renal cortex was centrifuged for 15 minutes at 4,500 revolutions per minute (rpm) in solution containing 0.25 M sucrose, 0.1 mM PMSF (phenylmethylsulfonylfluoride), a protease inhibitor, and 10 mM Tris HCl at pH 7.6. The supernatant was recentrifuged for 20 minutes at 14,000 rpm and the pellet resuspended. Percoll was added to the sucrose solution in a volume ratio of 1.0 ml Percoll per 11.5 ml sucrose solution. The mixture was centrifuged at 34,000 g for 35 minutes. The top, cloudy membrane band was separated from the dense pellet, resuspended in KCl solution, (100 mM KCl, 100 mM mannitol, and 5 mM (hydroxy-methyl)aminomethane-N-2-hydroxyethyl) pipperazine-N'-2-enthane-sulfonic acid (HEPES) pH 7.5 and centrifuged twice at 34,000 g for 30 minutes. The final membrane preparations were enriched five- to eightfold for (Na\(^+\), K\(^+\))-ATPase and were verified to be of proximal tubule origin by observing the response of adenyl cyclase activity to parathyroid hormone, isoproterenol, and arginine vaspressin as displayed in Figure 1. This pattern of hormone responsiveness has been shown previously to be characteristic of proximal tubule nephron segments [14, 15].

(Na\(^+\), K\(^+\))-ATPase activity was determined by the method of Quigley and Gottgerer [16] on 100 µl of membrane suspensions, following permeabilization by rapid freezing in dry ice/aceton and thawing, with absorbance determined at 740 nm (Beckman model 25 Spectrophotometer, Fullerton, California, USA). The reaction medium contained (mM): imidazole, 37.5; NaCl, 138; KCl, 19.0; Na ethyleneglycol-bis (b-aminoethylether-N, N'-tetraacetic acid (NaEGTA), 6.0; MgCl\(_2\), 5.0; and sodium azide, 6.0. The reaction was initiated by the addition of 4 mM Tris-ATP in the presence of 5 mM ouabain or an equal volume of deionized water. After 15 minutes at 37°C the reaction was terminated by addition of 50 µl cold 50% trichloroacetic acid. Total ATPase activity was estimated from the amount of inorganic phosphate (P\(_i\)) in the supernatant after centrifugation. (Na\(^+\), K\(^+\))-ATPase activity was measured as the difference between total ATPase and ouabain-insensitive (Na\(^+\), K\(^+\))-ATPase activities and was expressed as nmol P\(_i\) per milligram protein per minute. Protein content was determined by the method of Lowry et al with bovine serum albumin utilized as standard [17].

**Denervation**

Twenty-four-day old SHR and WKY rats were anesthetized with pentobarbital, 50 mg/kg body weight intraperitoneally. Following a midline abdominal incision, the left renal artery was isolated and surrounding tissue covered with 0.9% NaCl soaked gauze. The adventitia was then stripped from the artery under 4× magnification and continuously coated with a solution of 10% phenol in absolute alcohol for at least 10 minutes. Animals demonstrating spasm of the renal artery or blanching of the renal parenchyma were discarded. Following surgery the animals were allowed to recover over ten days while blood pressure and body weights were recorded. Rats that failed to gain weight over this period, as compared to age-matched, unoperated controls, were excluded. For paired comparison the (Na\(^+\), K\(^+\))-ATPase activity of BLMs from denervated kidneys were analyzed in respect to their appropriate contralateral, innervated kidneys. Only samples in which the denervated kidneys of one sample had individual tissue norepinephrine content of less than 10% of the contralateral kidney were considered adequately denervated and included in the results.

**Tissue norepinephrine content**

After removal of kidneys a coronal section of cortex was removed from the lower pole and frozen on dry ice. After weighing, the specimen was placed in 0.4 N perchloric acid with 5 mM reduced glutathione 10 ml/gram weight. Following homogenization of tissue the sample was centrifuged at 0°C to separate the supernatant. The supernatant was then diluted 1:10 with deionized water and stored at −70°C until analysis.

Tissue catecholamines (norepinephrine and epinephrine) were analyzed by the method of Hussain and Benedict [18] utilizing the transfer of the \(^3\)H-methyl group from 5-adenosyl-L-methionine-[\(^3\)H-methyl] to catecholamines in the presence of catechol-0-methyl-transferase and then isolated by thin layer chromatography. Samples were quantitated by scintillation counting (Packard Tri-Carb 460C, Downers Grove, Illinois, USA).
Table 1. Group characteristics—body weight and systolic blood pressure (BP)

<table>
<thead>
<tr>
<th></th>
<th>5-week-old WKY</th>
<th>5-week-old SHR</th>
<th>13-week-old WKY</th>
<th>13-week-old SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP mm Hg</td>
<td>113.7</td>
<td>118.9</td>
<td>115.8</td>
<td>185.2</td>
</tr>
<tr>
<td>SE</td>
<td>±6.8</td>
<td>±3.6</td>
<td>±4.0</td>
<td>±6.9</td>
</tr>
<tr>
<td>(N = animals)</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight g</td>
<td>75.5</td>
<td>54.6</td>
<td>238.7</td>
<td>238.0</td>
</tr>
<tr>
<td>SE</td>
<td>±3.5</td>
<td>±3.1</td>
<td>±1.4</td>
<td>±0.9</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Materials

Norepinephrine, isoproterenol, parathyroid hormone (Bovine 1-34), vanadate-free adenosine triphosphate, phenol, perchloric acid, and arginine vasopressin were obtained from Sigma Chemicals (St. Louis, Missouri, USA).

Statistical comparisons

Comparisons of (Na\(^+\), K\(^+\))-ATPase activity of the 5-, and 13-week old SHR and WKY were determined by analysis of variance with Dunnett’s multiple comparison test. (Na\(^+\), K\(^+\))-ATPase of denervated versus contralateral kidneys in the 5-week-old SHR and WKY were compared by paired Student’s t-test as the tissue for comparison was obtained from the same animal. Arterial blood pressure, body weight, and tissue catecholamine contents were compared by the Student’s t-test for unpaired data. Significance was defined by P < 0.05 for all data.

Results

The mean systolic arterial blood pressure and body weights are displayed in Table 1. There was no detectable difference in systolic blood pressure as determined at 28 to 32 days of age (5-week animals) but blood pressure increased significantly in SHR rats at 13 weeks of age. Thus, the studies performed at five weeks of age can be considered as representative of an early phase prior to the development of overt hypertension (“prehypertensive phase”). Similarly, while the 5-week SHR weighed less, weight was gained more rapidly so that there was no difference in weight between these strains at 13 weeks of age. Furthermore, unilateral denervation had no effect on blood pressure at 5 weeks in either WKY rats or SHR, (112.2 ± 4.7 vs. 111.0 ± 3.9 mm Hg).

To verify that alterations in proximal tubule (Na\(^+\), K\(^+\))-ATPase activity are present in SHR, (Na\(^+\), K\(^+\))-ATPase activity was determined in 5- and 13-week-old SHR and WKY rats. As shown in Figure 2, (Na\(^+\), K\(^+\))-ATPase activity in 5-week-old SHR was significantly greater than in age-matched WKY, 328.6 ± 18.9 versus 262.3 ± 34.6 nmoles P\(_1\)/mg protein/min. This observed increased BLM (Na\(^+\), K\(^+\))-ATPase activity was not present in 13-week-old SHR.

In order to test whether renal innervation was associated with the increased (Na\(^+\), K\(^+\))-ATPase activity at 5 weeks, SHR underwent unilateral denervation at 24 to 26 days of age. Renal proximal tubule BLMs were prepared at 5 weeks. The (Na\(^+\), K\(^+\))-ATPase activities of denervated kidneys were then compared to contralateral innervated kidneys. As shown in Figure 3, denervation decreased (Na\(^+\), K\(^+\))-ATPase activity from 316.8 ± 23.8 to 223.1 ± 23.9 nmoles P\(_1\)/mg protein - min (P < 0.02, ANOVA with Dunnett’s multiple comparison test).

Denervation of the kidney was verified by determination of tissue norepinephrine content and considered adequate if norepinephrine content was less than 10% of the contralateral kidney. Denervation was considered inadequate in nine animals and the mean NE content in these rats was 186.7 ± 61.9 (denervated) versus 204.9 ± 53.7 (contralateral) pg/mg kidney weight. In these animals, (Na\(^+\), K\(^+\))-ATPase activity was the same in the “denervated” and contralateral kidneys (Fig. 4), 363.2 ± 42.3 versus 358.9 ± 23.3 nmoles P\(_1\)/mg protein - min.

In eight animals, unilateral denervation in 5-week-old WKY, 354.8 ± 52.6 (WKY contralateral) versus 358.7 ± 102.3 (WKY denervated) nmoles P\(_1\)/mg - min (Fig. 4); or sham surgery in 5-week-old SHR (8 animals), 318 ± 70.8 (sham SHR) versus 328.6 ± 18.9 (control SHR) nmoles P\(_1\)/mg protein - min (Fig. 4), had no effect on renal BLM (Na\(^+\), K\(^+\))-ATPase activity. These data suggest that renal nerve integrity has no significant effect on (Na\(^+\), K\(^+\))-ATPase in WKY rats and that surgery has no effect on (Na\(^+\), K\(^+\))-ATPase. As such, these groups serve as negative controls for non-specific effects of surgery or pharmacologic agents on (Na\(^+\), K\(^+\))-ATPase activity.

Responsiveness of renal proximal tubule BLM (Na\(^+\), K\(^+\))-ATPase activity to catecholamines was studied by incubating cortical homogenates with norepinephrine, 1 µM, or vehicle for 15 minutes at 37°C while bubbled with 95% O\(_2\)/5% CO\(_2\). Following this incubation, BLMs from 5-week-old rats were prepared and (Na\(^+\), K\(^+\))-ATPase activity compared with BLMs from the same rats prepared identically except for incubation with control vehicle. This method of pre-treatment with catecholamines has been shown to stimulate (Na\(^+\), K\(^+\))-ATPase activity in rabbit renal proximal tubule BLMs whereas treat-
Fig. 3. \((Na^+, K^+)-ATPase\) activity of BLMs derived from denervated and contralateral kidneys of 5 week SHR. Points connect values derived from denervated and contralateral, innervated kidneys in the same rat. Mean values ± SEM are displayed for each group, \(P < 0.05\) by paired Student’s t-test.

Fig. 4. Pooled non-paired data for \((Na^+, K^+)-ATPase\) activity in BLMs enriched from renal cortex of denervated and contralateral kidneys of 5-week WKY rats, sham-operated 5-week SHR, and inadequately denervated SHR. The first set of bars represent \((Na^+, K^+)-ATPase\) activity of contralateral kidney (open bar) and denervated kidney (hatched bar) of WKY (\(N = 8\) animals). The middle set of bars represent \((Na^+, K^+)-ATPase\) activity of contralateral kidney (open bar) and sham-operated kidney (stippled bar) of SHR (\(N = 8\) animals). The last set of bars represent \((Na^+, K^+)-ATPase\) activity in unoperated (open bar) vs. inadequately denervated (solid bar) of SHR (\(N = 9\) animals). There is no difference in sodium pump activity in controls vs. experimental groups when data are pooled in this manner.

Table 2. Effect of norepinephrine (NE) on proximal tubule basolateral membrane \((Na^+, K^+)-ATPase\) activity in SHR and WKY rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>NE 1 (\mu)M</th>
<th>% Increase</th>
<th>(P) value (control vs. NE paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-week-old WKY</td>
<td>263.9 ± 36.0</td>
<td>339.6 ± 37.2</td>
<td>28.6 ± 4.6</td>
<td>(&lt;0.05)</td>
</tr>
<tr>
<td>5-week-old SHR</td>
<td>300.0 ± 35.7</td>
<td>420.7 ± 40.8</td>
<td>40.2 ± 4.2</td>
<td>(&lt;0.05)</td>
</tr>
</tbody>
</table>

Values given are means ± SEM, \(N = 6\) animals.

\(\ast\) \(P < 0.05\) percent increase from control as compared to 5-week-old WKY, unpaired Student’s t-test

The present results show that proximal tubule \((Na^+, K^+)-ATPase\) activity is greater during the pre-hypertensive phase in SHR than in age-matched WKY rats. Interestingly, this enhanced level of sodium pump activity is no longer apparent after hypertension has become established. Moreover, prior denervation of 5-week-old SHR mitigated the increased proximal tubule \((Na^+, K^+)-ATPase\) activity. In contrast, sham denervation of SHR or denervation of age-matched WKY rats had no effect on \((Na^+, K^+)-ATPase\) activity. Finally, exogenous norepinephrine induced a greater stimulatory effect on \((Na^+, K^+)-ATPase\) activity of BLMs derived from 5-week-old SHR than WKY rats.

Enhanced renal nerve activity or enhanced responsiveness to catecholamines have been suggested as possible mechanisms for the retention of sodium and the abnormal pressure-natriuresis relationship observed in animal models of genetic hypertension [7, 9, 20–22]. Both renal nerve stimulation and norepinephrine have been demonstrated to enhance renal proximal tubule sodium and water reabsorption, in vivo resulting in salt retention [6, 7, 23, 24]. Conversely, acute and chronic denervation leads to a natriuresis [25, 26]. Furthermore, norepinephrine has been shown to stimulate proximal tubule sodium reabsorption directly in vitro isolated, perfused tubules [12, 27]. Since norepinephrine has been demonstrated to stimulate \((Na^+, K^+)-ATPase\) activity in enriched basolateral membranes derived from rabbit proximal tubules [12], it seems reasonable to assume that the increase in sodium reabsorption may be due, in part, to enhanced \((Na^+, K^+)-ATPase\) activity.

Previous studies in genetic models of hypertension have supported more directly a role for the renal adrenergic system in the sodium retention which precedes the development of hypertension in SHR [7–11, 28, 29]. Firstly, more frequent efferent renal nerve impulses have been recorded in young SHR as opposed to WKY rats [3]. Secondly, renal denervation has
been shown to delay the onset and reduce the magnitude of hypertension in SHR [8, 10, 28, 29]. Moreover, additional studies have shown that the delay and reduction of hypertension in SHR by renal denervation is associated with higher sodium excretion rates [10, 11, 22]. Thirdly, avid sodium restriction retards and impairs the development of hypertension in SHR in a manner comparable to that observed after denervation [3]. Therefore, the association between renal nerve activity and the development of hypertension may be secondary to sodium retention per se. Though such findings support the view that renal nerve activity is involved primarily in the development of hypertension through sodium retention in SHR, the mechanism by which renal nerve activity augments sodium reabsorption and contributes to the development of hypertension is unknown.

The following findings relevant to the development of hypertension in SHR emerge from the present study. First, (Na⁺, K⁺)-ATPase activity in basolateral membranes enriched from proximal tubules of 5-week-old (prior to the development of hypertension) SHR is greater than that observed in age-matched WKY rats. Second, loss of renal nerve integrity during early development of hypertension significantly reduced proximal tubule BLM (Na⁺, K⁺)-ATPase activity to levels indistinguishable from the activity seen in WKY rats. Third, in vitro stimulation of BLM (Na⁺, K⁺)-ATPase activity in SHR was significantly greater than in WKY rats. The demonstration in this study of enhanced (Na⁺, K⁺)-ATPase activity in young SHR confirms findings reported previously by other investigators. For example, Garg, Narang and McArdle [30] showed that proximal tubule (Na⁺, K⁺)-ATPase activity in microdissected nephron segments was increased in 5-week-old SHR compared to WKY rats. In addition, this difference was no longer observed after the establishment of hypertension. Such findings are compatible with the results reported in the present study. The findings in the present study after unilateral denervation demonstrate for the first time the association between loss of renal nerve integrity and a reduction in proximal tubule (Na⁺, K⁺)-ATPase activity in pre-hypertensive SHR. Several alternative explanations for this relationship should be considered. First, the observed difference in proximal tubule (Na⁺, K⁺)-ATPase activity between the denervated and contralateral kidney in SHR could be the result of increased adrenergic receptor activity. Further clarification of this relationship and definition of the specific adrenergic receptor pathway are necessary. For example, alpha-2 adrenergic receptor denervation in SHR have demonstrated an increase in alpha-adrenergic receptor density with either no change or reduced receptor affinity [37–40]. In addition, alpha-receptor function is modulated by sodium intake. For example, alpha-2 adrenergic receptor density has been shown to increase in response to high sodium intake [39]. Furthermore, it was suggested that alpha-2 adrenergic receptor activity may contribute to renal sodium retention [41]. Conversely, DiBona and Sawin have presented evidence that suggests that renal nerve stimulation influences sodium transport through alpha-1 adrenergic receptor pathways [30]. The present findings suggest a relationship between adrenergic stimulation and increased sodium pump activity in SHR that is mediated by receptor function that may be additive to enhanced nerve traffic. However, further studies will be necessary to further clarify this relationship and define the specific adrenergic receptor pathways that are involved in these processes.

A final alternative explanation for the observed increased proximal tubule (Na⁺, K⁺)-ATPase activity in young SHR is that apical Na⁺ entry is enhanced. Increased Na⁺:H⁺ exchange has been demonstrated in 6-week-old SHR and may contribute

In 5-week-old WKY rats that underwent unilateral denervation, the levels of (Na⁺, K⁺)-ATPase activity in BLMS derived from the contralateral kidneys and denervated kidneys (Fig. 4) were equivalent to those observed in the SHR at the same age (Fig. 1). This observed equivalency in activity was due to a greater enrichment from the cortical homogenate (Na⁺, K⁺)-ATPase activity in the unilaterally denervated WKY rats (7.2-fold enrichment) than in the SHR (5.4-fold enrichment). This daily variability in enrichment was factored for in the age-related BLM (Na⁺, K⁺)-ATPase activity (Fig. 1) by identically preparing SHR and WKY matched kidneys on a daily basis (2 to 4 animals/day). This removes the variability in enrichment of BLMS because the predominant factors affecting enrichment are the conditions under which the basolateral membranes are prepared, such as time of preparation and subtle differences in suspension media and density gradients.

The present study also demonstrates that exogenously administered norepinephrine exerts a more pronounced stimulation of proximal tubule BLM (Na⁺, K⁺)-ATPase activity in SHR than in WKY. Since these studies were performed in vitro employing non-specific, but physiologic, agonist stimulation, such findings support the presence of enhanced adrenergic receptor activity. At present, it is widely accepted that alpha-adrenergic receptor activity is responsible for the transport effects of catecholamines on renal proximal tubules [7, 23, 35, 36]. Previous studies examining adrenergic receptor function in SHR have demonstrated an increase in alpha-adrenergic receptor density with either no change or reduced receptor affinity [37–40]. In addition, alpha-receptor function is modulated by sodium intake. For example, alpha-2 adrenergic receptor density has been shown to increase in response to high sodium intake [39]. Furthermore, it was suggested that alpha-2 adrenergic receptor activity may contribute to renal sodium retention [41]. Conversely, DiBona and Sawin have presented evidence that suggests that renal nerve stimulation influences sodium transport through alpha-1 adrenergic receptor pathways [30]. The present findings suggest a relationship between adrenergic stimulation and increased sodium pump activity in SHR that is mediated by receptor function that may be additive to enhanced nerve traffic. However, further studies will be necessary to further clarify this relationship and define the specific adrenergic receptor pathways that are involved in these processes.
to sodium retention [42]. Several lines of evidence, however, support a more direct effect of catecholamines on (Na$^+$, K$^+$)-ATPase activity as opposed to apical sodium entry. First, (Na$^+$, K$^+$)-ATPase activity in permeabilized basolateral membranes (in vitro sodium concentration is controlled) derived from cortical homogenates incubated with norepinephrine is increased in SHR and WKY rats. In this regard, induction of (Na$^+$, K$^+$)-ATPase activity in nephron segments due to enhanced apical sodium entry appears to require more than three hours [43], whereas the preparation of basolateral membranes and measurement of (Na$^+$, K$^+$)-ATPase activity in the present experiments was accomplished over two hours. Second, catecholamines increase transport-dependent oxygen consumption, and, thus, (Na$^+$, K$^+$)-ATPase activity, in rabbit proximal tubules in which the gradient for Na$^+$ entry has been negated to retard sodium entry or in permeabilized cells in which Na$^+$ entry has been enhanced [12]. These data would support the view that a combination of enhanced sodium entry through Na$^+$:H$^+$ exchange and Na$^+$ exit through (Na$^+$, K$^+$)-ATPase activity may combine to promote sodium retention in young SHR.

Overall, the relationships between proximal tubule (Na$^+$, K$^+$)-ATPase activity, renal nerve integrity, and the development of hypertension is indirectly supported by the following observations. First, renal denervation early in the development of hypertension of SHR (at a period during which proximal tubule (Na$^+$, K$^+$)-ATPase activity is increased) significantly delays and reduces the level of hypertension [8, 10, 28, 29]. Second, renal denervation decreases the proximal tubule (Na$^+$, K$^+$)-ATPase activity to levels comparable to the activity observed in age-matched genetic control rats. Third, renal denervation after the establishment of hypertension (at a time during which (Na$^+$, K$^+$)-ATPase activity is not increased) has little to no effect on blood pressure [30]. These observations taken together suggest that the renal adrenergic system may contribute to the development of hypertension but other primary or secondary factors, such as an endogenous sodium pump inhibitor, contribute to the maintenance phase of hypertension in SHR. Speculatively, the sodium retention by adrenergic mechanisms could be a stimulus for these potential maintenance factors.

In conclusion, the present results substantiate a relationship between renal nerve integrity and adrenergic receptors in the modulation of renal proximal tubule (Na$^+$, K$^+$)-ATPase activity. It seems reasonable to conclude, therefore, that adrenergic mechanisms may increase proximal tubule (Na$^+$, K$^+$)-ATPase activity prior to the development of hypertension in SHR. If correct, it would follow that increased sodium pump activity may then contribute to renal sodium retention and, thus, to the development of hypertension in this genetic model of hypertension.

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