Ontogenesis of Sympathetic Responsiveness in Spontaneously Hypertensive Rats

I. Renal $\alpha_1$, $\alpha_2$, and $\beta$-Adrenergic Receptors and Their Signaling

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We studied the ontogenetic development of renal $\alpha_1$, $\alpha_2$, and $\beta$-adrenergic receptors and their coupling to inositol phosphate and cyclic AMP formation in spontaneously hypertensive and normotensive Wistar-Kyoto rats. $\alpha_1$, $\alpha_2$, and $\beta$-Adrenergic receptor number was significantly increased in hypertensive compared with normotensive rats, but the increase did not precede blood pressure elevation. Despite increased $\alpha_1$-adrenergic receptors, basal and norepinephrine-stimulated inositol phosphate formation remained unchanged in all age groups. Rat kidney contains $\alpha_1$- and $\alpha_2$-adrenergic receptors coupling to inositol phosphate formation by different mechanisms, but the relative contribution of $\alpha_1$- and $\alpha_2$-adrenergic receptors to norepinephrine-stimulated inositol phosphate formation was similar in normotensive and hypertensive rats. Despite increased $\beta$-adrenergic receptors, basal, isoproterenol-, and forskolin-stimulated cyclic AMP accumulation was similar in normotensive and hypertensive rats. We conclude that the number but not the functional responsiveness of renal adrenergic receptors increases in spontaneously hypertensive rats. Thus, the additional receptors are unlikely to contribute to the pathophysiology of elevated blood pressure in this model. (Hypertension 1993;22:169-177)

KEY WORDS • receptors, adrenergic • inositol phosphates • adenosine cyclic monophosphate • kidney • rats, inbred SHR

The kidney is the tissue with the greatest long-term effect on blood pressure control. Cross-transplantation studies have demonstrated that transplantation of the kidney of a genetically hypertensive rat can elevate blood pressure in immunotolerant recipients, whereas transplantation of the kidney of a normotensive animal can lower blood pressure in immunotolerant borderline hypertensive rats. The adrenergic system is a major mechanism for the neural and hormonal control of renal function. This occurs extrarenally by the well-known alterations of cardiac and vascular function that affect efferent renal nerves via baroreceptor and volume reflexes. Perhaps even more important are intrarenal effects of the sympathoadrenal system, which can occur by alterations of renin release, renal blood flow, glomerular filtration rate, and tubular function. Thus, the kidney is richly endowed with adrenergic receptors. In the rat, renal $\alpha$-adrenergic receptors are located mainly postsynaptically, but some may also exist presynaptically. Rat renal $\alpha_2$-adrenergic receptors have been demonstrated presynaptically, but extrajunctional $\alpha_2$-adrenergic receptors have also been found. Some renal $\beta$-adrenergic receptors are located presynaptically, but many are found on tubular cells. Based on these considerations, many studies have investigated possible alterations of renal adrenergic receptors in states of elevated blood pressure and their possible pathophysiological role in this disease state. Thus, it has been found repeatedly that the number of renal $\alpha_1$-adrenergic receptors is increased in genetically hypertensive rats of the Kyoto spontaneously hypertensive rat (SHR) strain but also of some other strains and that this increase is specific for the genetic models of hypertension. Studies on renal $\alpha_2$-adrenergic receptors in SHR have been performed using $[^3H]$yohimbine or $[^3H]$rauwolscine as the ligand that labels homogeneous populations of $\alpha_2$-adrenergic receptors and fails to detect the minor population of $\alpha_2$-adrenergic receptors that is also present in rat kidney. Elevations of renal $\alpha_2$-adrenergic receptors have also been found specifically in SHR, but this finding was somewhat less consistent. Such studies were performed using $[^3H]$prazosin or $[^3H]$BE-2254 as the ligand that labels total $\alpha_2$-adrenergic receptors; rat kidney contains both $\alpha_2$- and $\alpha_2$-adrenergic receptors, but two recent studies have demonstrated that the ratio of the two subtypes remains unchanged in SHR when compared with normotensive Wistar-Kyoto (WKY) rats. Elevations of both $\alpha_1$- and $\alpha_2$-adrenergic receptor numbers have been reported to precede the development of hypertension. Based on these findings, we have previously hypothesized that genetically determined alterations of renal $\beta$-adrenergic receptors might play an important pathophysiological role in the development of hypertension in the SHR. More recent studies in our labora-
Animals

WKY rats and SHR were obtained from Mollegard, Skensved, Denmark. Male and female rats of each strain were interbred to generate male progeny that were killed at the ages of 3, 6, and 8 weeks by cervical dislocation without prior anesthesia to avoid anesthesia-induced activation of the sympathetic nervous system. After breeding was complete, male breeders were killed at the age of 28 weeks. One day before death, systolic blood pressure measurements in the established phase of hypertension. Therefore, we have studied α- and β-adrenergic receptor signaling in relation to the ontogenetic development of hypertension in WKY and SHR kidneys. Our data demonstrate that increased renal α- and β-adrenergic receptors are not associated with enhanced signaling and thus provide further evidence against an important pathophysiological role of alterations in renal adrenergic receptors in the SHR model of hypertension.

Methods

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Radioligand Binding

Radioligand binding to α- and β-adrenergic receptors was performed as previously described.17 Briefly, renal membranes were prepared in a glass homogenizer with a motor-driven PTFE (Teflon) pestle (1500 rpm, 10 strokes) followed by centrifugation at 50,000g for 20 minutes at 4°C. This method yields a low receptor density when expressed in milligrams per milliliter of protein but has the advantage of making all receptors present in the tissue available to the ligand; more refined membrane preparations usually suffer from considerable loss in yield. The pellet was resuspended in binding buffer (50 mmol/L Tris-HCl and 0.5 mmol/L EDTA, pH 7.5, for α-adrenergic receptors; 154 mmol/L NaCl and 10 mmol/L Tris-HCl, pH 7.4, for β-adrenergic receptors). Experiments were performed in a total volume of 1000 μL (α-adrenergic receptors) or 250 μL (α- and β-adrenergic receptors) containing approximately 200 (α1), 100 (α2), or 25 (β) μg of protein per assay. α1-Adrenergic receptors were labeled with [3H]prazosin, α2-adrenergic receptors with [3H]rauwolscine, and β-adrenergic receptors with [3H]iodocyanopindolol. The mixtures were incubated at 25°C (α-adrenergic receptors) or 37°C (β-adrenergic receptors) for 45 (α1-adrenergic receptors), 60 (α2-adrenergic receptors), or 90 (β-adrenergic receptors) minutes. The incubations were stopped by rapid vacuum filtration over Whatman GF/C filters and two subsequent washes with 10 mL each of binding buffer. Nonspecific binding was defined as binding in the presence of 10 μmol/L phenolamine (α-adrenergic receptors) or 1 μmol/L of the hydrophilic antagonist (±)-CGP 12177 (β-adrenergic receptors). The affinity (Kd) and number (Bmax) of binding sites were determined from the experimental data with plots according to Scatchard.22

Inositol Phosphate Measurements

Inositol phosphate accumulation was determined in renal cortical slices (350×350 μm) as described previously. Briefly, the slices were resuspended in Krebs-Henseleit buffer of the following composition (mmol/L): NaCl, 118; KCl, 4.7; CaCl2, 1.3; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 24.9; glucose, 11; and EDTA, 0.001. The buffer was supplemented with 10 mmol/L LiCl to block inositol phosphate degradation, 2 μM adenosine deaminase to remove adenosine liberated during tissue chopping from the assay, and 20 μmol/L cocaine to block neuronal catecholamine uptake. After two washes with fresh buffer during a 30-minute incubation at 37°C, the slices were labeled for 60 minutes with 100 μCi of [3H]myo-inositol per 11 mL of suspension containing 150 to 300 μg of slices. Then, 300-μL aliquots of the suspension were pipetted into flat-bottomed polystyrene tubes under gentle swirling, and agonists and antagonists were added to yield a final volume of 330 μL. After 45 minutes in the presence of agonists and antagonists, the incubation was stopped by addition of 330 μL methanol and 600 μL chloroform. After vigorous mixing, the phases were separated by centrifugation at 5200g for 10 minutes at 4°C, and aliquots (450 μL) of the upper phase were placed on Dowex AG 1-8X columns (200 mg per column). After elution of free inositol with twice 5 mL H2O and twice 5 mL of 60 mmol/L ammonium formate, total inositol phosphates were eluted by addition of twice 1 mL of 1 mol/L ammonium formate dissolved in 100 mmol/L formic acid. The recovered radioactivity was determined in a scintillation counter at 42% efficiency.

[3H]Inositol incorporation was assessed by counting the radioactivity in aliquots of the inositol-loaded slice suspension after three washes with buffer. [3H]Inositol phosphate formation was expressed as the percentage of incorporated [3H]inositol.

Cyclic AMP Measurements

Renal cortical slices of 350×350 μm were prepared with a tissue chopper (Bachofer, Reutlingen, Germany). Slices were washed four times with fresh Krebs-Henseleit buffer (supplemented with 2 U/mL adenosine deaminase to inactivate endogenous adenosine) during a 1-hour incubation at 37°C. Slices were aliquoted (approximately 15 to 30 mg wet weight per assay) and incubated in a total volume of 500 μL containing 100 μmol/L isobutylmethylxanthine to inhibit phosphodiesterases for 10 minutes at 37°C; additionally, some tubes contained 100 μmol/L isoproterenol or 10 μmol/L forskolin. The incubation was stopped by addition of 500 μL stopping buffer (2% sodium dodecyl sulfate and 50 mmol/L Tris, pH 7.5) and boiling...
for 5 minutes. The boiled samples were centrifuged at 13 000g for 5 minutes, and the supernatants were placed on neutral alumina columns. The columns were eluted with 1 mL of 100 mmol/L Tris at pH 7.5, and the cyclic AMP (cAMP) content in the eluate was determined with a commercially available radioimmunoassay (NEN, Dreieich, Germany). Recovery from the columns was typically 80%. The amount of cAMP accumulated in each sample was calculated and normalized for wet weight.

Chemicals

[3H]Prazosin (specific activity, approximately 80 Ci/mmol), [3H]rauwolscine (specific activity, approximately 80 Ci/mmol), and [3H]iodocyanopindolol (specific activity, 2200 Ci/mmol) were obtained from NEN; [3H]m>[o-inositol (specific activity, 80 to 120 Ci/mmol, prepurified with PT6-271) was from Amersham, Braunschweig, Germany. Pretolamine and (+)-CGP 12177 (l-[2-(3-carbamoyl-4-hydroxy)phenoxyethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxyl]-2-propanol methanesulfonate) were kind gifts of CIBA-GEIGY, Basel, Switzerland, and (+)-niguldipine of BYK-Gulden, Konstanz, Germany.

Data Evaluation

All data are mean±SEM of n experiments. Statistical significance of differences between groups was assessed by two-tailed unpaired t tests when two groups were compared and by one-way analysis of variance when multiple groups were compared. If one-way analysis of variance demonstrated that the variance between groups was significantly greater than that within groups, individual groups were compared by t tests with Bonferroni corrections for multiple comparisons. All statistical calculations were performed with the INSTAT program (GraphPad Software, San Diego, Calif). A value of P<.05 was considered significant.

Results

Physiological Parameters

As expected, systolic blood pressure increased only slightly during development in WKY rats but increased markedly in SHR (Table 1). Thus, starting at 6 weeks of age, systolic blood pressure was significantly elevated in SHR compared with age-matched WKY rats. For technical reasons, we were not able to determine systolic blood pressure in 3-week-old rats, but it is well documented that 3-week-old SHR have only marginal if any blood pressure elevations compared with WKY rats20,26; thus, we refer to the 3-week-old age group as prehypertensive in this article. Body and renal weights also increased with age but did not differ between strains, with the exception of the 3-week-old age group, in which both were significantly reduced (Table 1). Because the delay in body weight gain exceeded that in renal weight gain, a slight but significant renal hypertrophy (assessed as renal weight–body weight ratio) was detected in 3-week-old SHR (Table 1).

Table 1. Development of Systolic Blood Pressure, Body Weight, and Renal Weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 Weeks</th>
<th>6 Weeks</th>
<th>8 Weeks</th>
<th>28 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>n.d.</td>
<td>82±3</td>
<td>122±2*</td>
<td>190±4*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>36±1</td>
<td>28±1*</td>
<td>119±4</td>
<td>111±4</td>
</tr>
<tr>
<td>Kidney weight (mg)</td>
<td>191±7</td>
<td>170±5†</td>
<td>523±16</td>
<td>765±15</td>
</tr>
<tr>
<td>Kidney/body weight (g/kg)</td>
<td>5.3±0.1</td>
<td>6.2±0.1*</td>
<td>4.4±0.1</td>
<td>4.0±0.1</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyto rats; SHR, spontaneously hypertensive rats; n.d., not determined for technical reasons. Data are mean±SEM of the number of animals indicated in parentheses.

*P<.001, †P<.05 compared with age-matched WKY rats.

FIG 1. Line graph shows development of α1-adrenergic receptor density determined in renal membranes of Wistar-Kyoto (WKY) rats (○) and spontaneously hypertensive rats (SHR) (●) from [3H]prazosin saturation binding experiments. Data are mean±SEM of eight rats per age group. *P<.05 vs age-matched WKY rats.
we performed experiments on norepinephrine-stimulated inositol phosphate formation in renal cortical slices. Incorporation of \(^{3}H\)myo-inositol was similar in all age groups of both strains and typically ranged between 6000 and 20 000 cpm/mg wet wt (data not shown). Basal inositol phosphate formation was lowest at 6 weeks (approximately 0.6% of incorporated radioactivity) and highest at 28 weeks (approximately 2.2% of incorporated radioactivity, \(P<.001\) vs 6 weeks), with intermediate values in the other two groups. However, we did not detect significant differences between strains in any age group (data not shown). Norepinephrine-stimulated inositol phosphate formation (expressed as percent over basal) was tested at agonist concentrations of 1 and 100 \(\mu\)M. Data are expressed as percentage of inositol phosphates formed above basal, with basal values being significant in individual age groups. When the data of all rats and SHR, we performed power calculations. These was not significantly different between strains at any age error regarding the lack of differences between WKY used (Fig 2). To estimate the possibility of a type II error regarding the lack of differences between WKY rats and SHR (hatched bars) during a 45-minute incubation in the presence of 10 mmol/L LiCl in the absence and presence of 100 mmol/L (+)-niguldipine. Data are shown as percent inhibition by (+)-niguldipine and reflect the relative contribution of \(\alpha_{1A}\) -adrenergic receptors to the inositol phosphate response. Data are mean±SEM of 8 to 10 experiments in each age group.

hypertensive age groups were pooled, an alteration of at least 15% would have been detected as statistically significant, with an \(\alpha\) of 0.05 and \(\beta\) of 0.05.

Although the ratio of renal \(\alpha_{1A}\)- to \(\alpha_{1B}\)-adrenergic receptor number remains unchanged in SHR compared with WKY rats,\(^{17,18}\) the different pathways used by both receptor subtypes to couple to inositol phosphate formation in rat kidney? opened the possibility that the contribution of both subtypes to norepinephrine-stimulated inositol phosphate formation might be altered in SHR. To test whether differential regulations of \(\alpha_{1A}\) and \(\alpha_{1B}\)-adrenergic responsiveness might offset each other, we determined the inhibition of norepinephrine-stimulated inositol phosphate formation by 100 nmol/L of the highly \(\alpha_{1A}\)-selective antagonist (+)-niguldipine\(^{28}\) in 3- and 28-week-old WKY rats and SHR. This concentration of (+)-niguldipine was chosen because it occupies more than 92% of \(\alpha_{1A}\)- and less than 2% of \(\alpha_{1B}\)-adrenergic receptors as calculated based on its affinities\(^{17,18}\) after correction for the presence of 100 \(\mu\)mol/L norepinephrine based on its potency in this system (EC\(_{50}\), 3.1 \(\mu\)M). (+)-Niguldipine inhibited norepinephrine-stimulated inositol phosphate formation to a similar extent in 3- and 28-week-old WKY rats and SHR (Fig 3). Moreover, there was no significant difference between WKY rats and SHR with regard to stimulation of inositol phosphate formation by the somewhat \(\alpha_{1A}\)-selective agonist methoxamine in 6- and 8-week-old rats (data not shown).

**\(\alpha_{2}\)-Adrenergic Receptors**

The affinity of \(^{3}H\)rauwolscine for rat renal \(\alpha_{2}\)-adrenergic receptors ranged between 3.3 and 4.3 nmol/L and did not vary significantly between strains or age groups (data not shown). The density of \(\alpha_{2}\)-adrenergic receptors in young and adolescent WKY rats ranged between 42 and 49 fmol/mg protein and significantly increased in adult WKY rats (72±5 fmol/mg protein, \(P<.05\), Fig 4). In contrast, renal \(\alpha_{2}\)-adrenergic
receptor density gradually increased in SHR from 55±8 fmol/mg protein at 3 weeks to 90±5 fmol/mg protein at 28 weeks (P<.05 for 28 vs 3 or 6 weeks, Fig 4). In all hypertensive age groups, the renal α1-adrenergic receptor density was significantly greater in SHR than in age-matched WKY rats, whereas we did not detect a significant difference for prehypertensive SHR (Fig 4).

To determine the functional relevance of increased α1-adrenergic receptors in SHR kidney, we tried to measure inhibition of cAMP accumulation in renal cortical slices from WKY rats and SHR. Regardless of whether forskolin (10 μmol/L) or parathyroid hormone (100 nmol/L) was used to stimulate cAMP formation, we were unable to obtain consistent inhibition of cAMP formation by the α1-adrenergic agonists epinephrine, α-methyl-norepinephrine, bromoxindole, moxonidine, or azepexole (all in the presence of 10 μmol/L [±]propranolol to block β-adrenergic receptors, data not shown). In contrast, epinephrine (100 μmol/L) in the presence of 10 μmol/L [±]propranolol) consistently enhanced cAMP formation in the presence of 10 μmol/L forskolin, which might indicate the presence of atypical (propranolol-insensitive) "β2-like" adrenergic receptors in rat kidney. This stimulatory effect of epinephrine was similar in WKY rats and SHR at 6 and 8 weeks of age (data not shown) and was not further investigated in the present study.

**β-Adrenergic Receptors**

[125I]Iodocyanopindolol bound to rat renal β-adrenergic receptors with affinities ranging between 10 and 40 pmol/L that did not vary significantly between strains or age groups (data not shown). β1-Adrenergic receptor density ranged from 8 to 13 fmol/mg protein in WKY kidneys and from 12 to 16 fmol/mg protein in SHR kidneys (Fig 5). In all hypertensive age groups, it was higher in SHR than in WKY rats, although this difference did not reach statistical significance in the 8-week-old group (Fig 5).

To test the functional relevance of β-adrenergic receptor elevations in hypertensive SHR, we investigated cAMP accumulation in renal cortical slices. Basal cAMP accumulation was approximately 20 pmol per 10 minutes per 100 mg wet weight. This value was similar in all age groups and did not significantly differ between strains (Fig 6). Isoproterenol (100 μmol/L) increased cAMP accumulation by approximately 40%; this enhancement was similar in all age groups and in WKY rats and SHR (Fig 6). Power calculations demonstrated that with the given sample size and variance our study would have detected alterations of 25% to 50% in isoproterenol-stimulated cAMP accumulation as statistically significant when individual age groups were analyzed. When pooled data from all hypertensive age groups were analyzed, our study would have detected a 25% alteration as statistically significant, with an α of 0.05 and β of 0.2.

Parathyroid hormone (100 nmol/L) increased cAMP formation in 3- and 28-week-old WKY rats and in 3-week-old SHR by approximately 20%; in 28-week-old SHR, however, parathyroid hormone failed to significantly stimulate cAMP formation (Table 2). Forskolin (10 μmol/L) increased cAMP accumulation much more efficiently (by approximately 350%) than the hormone receptor agonists, but forskolin-stimulated cAMP formation did not differ significantly among age groups or strains (Fig 6).

**Discussion**

A rich sympathetic innervation, a large complement of adrenergic receptors, and a huge body of biochemical and physiological data demonstrate the important role of the sympatoadrenal system in the regulation of renal function. Both the sympathetic nervous system and the kidney have frequently been implied in theories regarding the development of elevated blood pressure in SHR or hypertensive patients.121,29,30 So many laboratories have studied possible alterations of renal α- and β-adrenergic receptors in SHR and other rat models of hypertension.13,14 The evolving consensus of such studies is that renal α1- and α2-receptors, and β-adrenergic receptor numbers increase in SHR with established hypertension. Elevated α2- and β-adrenergic receptors were found in almost all studies; the increase in renal α1-adrenergic receptors is somewhat less consistent, but reduced renal α1-adrenergic receptors were not reported. The elevations of renal α1- and α2-adrenergic receptors are specific for SHR and possibly some other models of genetic hypertension, because unchanged or even reduced α1- and α2-adrenergic receptors are found...
in rat models with acquired hypertension. Despite this specificity, increased renal \( \alpha_1 \)-, \( \alpha_2 \)-adrenergic receptors, or both do not appear to be directly involved in the pathogenesis of blood pressure elevations in the SHR. Renal \( \beta \)-adrenergic receptors are elevated in various forms of genetic and acquired hypertension, and the magnitude of the receptor increase appears to correlate with that of the blood pressure elevation. Thus, the regulation of renal \( \beta \)-adrenergic receptors appears to be closely linked to that of blood pressure but does not appear to be specific for genetic hypertension.

It is now clear that the three major classes of adrenergic receptors, ie, \( \alpha_1 \), \( \alpha_2 \), and \( \beta \), all exist in multiple subtypes, and the possibility exists that the subtypes undergo differential regulation in hypertension. Rat kidney expresses both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors, but their ratio remains unchanged in SHR. Ligands such as [\( ^3H \)]rauwolscine or [\( ^3H \)]yohimbine detect only \( \alpha_2 \)-adrenergic receptors in rat kidney, but Northern blot analysis and binding with [\( ^3H \)]RX 821002 as the ligand suggest that a minor population of \( \alpha_2 \)-adrenergic receptors also exists; studies on renal \( \alpha_2 \)-adrenergic receptors in hypertension, however, have always used rauwolscine or yohimbine as the ligand. Rat kidney expresses \( \beta_1 \) - and \( \beta_2 \)-adrenergic receptors, but their ratio remains constant during the development of SHR and in various other models of hypertension. Thus, it can be concluded on the basis of previously published data that alterations in the number of \( \alpha_1 \)-, \( \alpha_2 \)-, \( \beta_1 \)-, or \( \beta_2 \)-adrenergic receptors in established hypertension are unlikely to have a causative effect on blood pressure. Therefore, the present study has investigated two questions regarding a possible role of adrenergic receptor alterations for the pathophysiology of SHR: What is the relation between increased adrenergic receptors and blood pressure over time? Does the increased adrenergic receptor number translate into altered receptor responsiveness?

Few previous studies have investigated renal adrenergic receptors in very young SHR. Sanchez et al have compared the ontogenesis of renal \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors in WKY rats and SHR and detected significant increases in both receptors in SHR at 5 and 8 weeks and in all older age groups but not at 1, 4, 6, or 7 weeks of age. Although systolic blood pressure was higher in SHR than in WKY rats at all age groups in that study, this difference reached statistical significance only starting at 8 weeks with the given sample size. Fukuda et al detected significantly elevated renal \( \alpha_1 \)-adrenergic receptors in 4-week-old SHR but did not report blood pressure values for these animals. The present study failed to detect significant alterations of \( \alpha_1 \) - or \( \alpha_2 \)-adrenergic receptors in 3-week-old SHR. We have previously reported significant elevations (29%) of renal \( \beta \)-adrenergic receptors in 5- to 6-week-old SHR; although the present study confirmed this finding in 6-week-old SHR, it did not detect such increases in 3-week-old SHR. Thus, increased renal adrenergic receptors are not consistently found in very young SHR. Because increases in peripheral resistance have been detected even in very early ages of the SHR with the use of appropriately sensitive techniques and sample sizes, we conclude that increased renal adrenergic receptors are unlikely to precede blood pressure elevations in SHR; if anything, they accompany such elevations and may be part of the response to a small but physiologically important blood pressure increase, sodium retention, or both.

Because receptor number and responsiveness do not always correlate, a more important question is whether increased adrenergic receptor numbers translate into increased functional responsiveness in SHR kidney. The

**Table 2. Parathyroid Hormone-Stimulated Cyclic AMP Accumulation**

<table>
<thead>
<tr>
<th>Age</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Weeks (n=8)</td>
<td>28±13</td>
<td>20±13</td>
</tr>
<tr>
<td>28 Weeks (n=10)</td>
<td>25±5</td>
<td>−10±6*</td>
</tr>
</tbody>
</table>

WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. Data are mean±SEM of 100 nmol/L parathyroid hormone-stimulated cyclic AMP accumulation expressed as percent over basal (shown in Fig 6).

*P<.01 vs age-matched WKY rats.
first step in renal $\alpha_2$-adrenergic receptor signaling is the formation of inositol phosphates.\textsuperscript{32} Despite significantly increased $\alpha_1$-adrenergic receptor number, we failed to detect enhanced norepinephrine-stimulated inositol phosphate formation. It could be argued that the maximal inositol phosphate formation was unchanged despite receptor increases, because something other than receptor number is limiting inositol phosphate formation. This is unlikely to be the case, because the inositol phosphate response to submaximally effective norepinephrine concentrations was also unchanged in our study. Moreover, we have previously demonstrated in kidneys from adult SHR that the $EC_{50}$ for norepinephrine-stimulated inositol phosphate formation was not altered, whereas the maximal stimulatory effect was reduced.\textsuperscript{33} Reduced inositol phosphate formation accompanied by unchanged $\alpha_1$-adrenergic receptor numbers has also been found by other investigators.\textsuperscript{34} Whereas these three studies differ with regard to the presence of increased $\alpha_1$-adrenergic receptor number and reduced inositol phosphate formation, they all demonstrate that the amount of inositol phosphates formed per $\alpha_1$-adrenergic receptor is reduced in hypertensive SHR; thus, they agree that renal $\alpha_1$-adrenergic receptor function is not enhanced in SHR.

Because both $\alpha_1A$- and $\alpha_1B$-adrenergic receptors couple to inositol phosphate formation in rat kidney\textsuperscript{25-35} but use different pathways to do so,\textsuperscript{27} it could be possible that opposite changes in the responsiveness of both subtypes offset one another. We have previously demonstrated that the ratio of renal $\alpha_1A$- to $\alpha_1B$-adrenergic receptors remains unchanged in SHR.\textsuperscript{17,18} The present study demonstrates that the same is true for the contribution of both subtypes to norepinephrine-stimulated inositol phosphate formation in 3- and 28-week-old SHR. Moreover, the stimulatory effects of the $\alpha_1A$-selective agonist methoxamine\textsuperscript{36} were also unchanged in 6- and 8-week-old SHR (data not shown).

Thus, increased or unchanged renal $\alpha_2$-adrenergic receptors in SHR translate into unchanged or even decreased signaling of these receptors. These data give further evidence against the previous hypothesis\textsuperscript{31} that alterations in number or coupling efficiency of renal $\alpha_2$-adrenergic receptors are important for the development of hypertension in the SHR. This idea is further supported by recent data demonstrating that the reductions in urine formation and fractional and absolute sodium excretion caused by renal nerve stimulation and mediated by $\alpha_2$-adrenergic receptors\textsuperscript{3} are similar in extent in WKY rats and SHR.\textsuperscript{37} Moreover, continuous treatment with the $\alpha_2$-adrenergic antagonist terazosin\textsuperscript{38} has been demonstrated not to prevent the development of hypertension in SHR.\textsuperscript{39} On the other hand, increased afferent and efferent arteriolar vasoconstrictive responsiveness to $\alpha_2$-adrenergic stimulation has recently been reported,\textsuperscript{6} but in light of the above findings, we suggest that this is related to structural vessel alterations (see below) rather than to a specific alteration in $\alpha_2$-adrenergic responsiveness.

Studies in SHR aorta have suggested that the basal activity of one or more phospholipase C isozymes may be increased in SHR.\textsuperscript{14} Studies in resistance vessels\textsuperscript{59} and the present and previous data\textsuperscript{13,34} in kidney do not confirm an enhanced basal inositol phosphate formation and thus indicate a tissue-specific regulation of phospholipase C activity in SHR. Multiple allelic polymorphisms of phospholipase C isozyme genes have been detected in SHR compared with WKY rats,\textsuperscript{60,61} but only one polymorphism in the phospholipase C-$\delta$ gene appears to be specific for genetic forms of hypertension. Although this polymorphism encodes a point mutation in the catalytic domain of the enzyme,\textsuperscript{41} it is not clear whether this yields altered enzymatic activity. In fact, a recent breeding study demonstrated that the SHR allele of phospholipase C-$\delta$ cosegregates with low rather than with high blood pressure in an F\textsubscript{2} population of WKY $\times$ SHR hybrid rats.\textsuperscript{42} Because increased wall stress, eg, caused by enhanced pressure or by osmotic factors, can also activate phospholipase C,\textsuperscript{43} we therefore speculate that such effects rather than genetic factors could explain the enhanced basal inositol phosphate formation sometimes seen in some SHR vessels.

An elevation of renal $\alpha_1$-adrenergic receptors in SHR is one of the most consistent findings in studies of the sympathoadrenal system in hypertension.\textsuperscript{13,14} This appears to be important because $\alpha_1$-adrenergic receptors outnumber all other forms of adrenergic receptors in rat kidney. However, surprisingly little is known regarding the functional role of renal $\alpha_1$-adrenergic receptors in the rat. They have been demonstrated to inhibit renin release,\textsuperscript{44,45} functionally antagonize the actions of vasopressin in collecting ducts,\textsuperscript{46} and to promote intrarenal vasoconstriction in conscious rats,\textsuperscript{5} but the major-ity of rat renal $\alpha_2$-adrenergic receptors are located in proximal tubules.\textsuperscript{47} Tubular $\alpha_2$-adrenergic receptors couple to enhanced sodium uptake\textsuperscript{48,49} and oxygen consumption,\textsuperscript{50} but it remains unclear how this relates to overall renal sodium excretion. Because $\alpha_2$-adrenergic receptors couple to inhibition of cAMP formation in all known model systems,\textsuperscript{51} we tried to study their function at this level of the signaling pathway. Previous studies have detected inhibition of cAMP formation by $\alpha_2$-adrenergic agonists in some renal preparations but not in others.\textsuperscript{44} Unfortunately, we were not able to detect inhibition of cAMP formation in renal cortical slices in normotensive or hypertensive rats despite the use of various chemically distinct $\alpha_2$-adrenergic agonists. Because no other studies have reported on $\alpha_2$-adrenergic function in SHR kidney, it remains unclear whether increased $\alpha_1$-adrenergic receptor numbers are accompanied by enhanced responsiveness despite the huge number of rat renal $\alpha_2$-adrenergic receptors.

Finally, we have investigated the coupling of renal $\beta$-adrenergic receptors to cAMP formation in the SHR. Previous data have reported enhanced,\textsuperscript{52} unchanged,\textsuperscript{53} and reduced\textsuperscript{54} cAMP formation in kidneys of hypertensive rats after stimulation by isoproterenol; our study did not detect altered cAMP formation. An unaltered cAMP formation despite elevated renal $\beta$-adrenergic receptors in this and various previous studies\textsuperscript{13} indicates the existence of a disturbed effector coupling of $\beta$-adrenergic receptors and perhaps other $\mathrm{G}_\text{i}$-like hormone receptors. This idea is supported by the desensitized cAMP formation in response to parathyroid hormone in hypertensive SHR in this and a previous study,\textsuperscript{55} although interpretation of the parathyroid hormone data is hampered by lack of data regarding a possible alteration in receptors for this hormone in SHR kidney and by lack of knowledge regarding a possible colocalization of $\beta$-adrenergic receptors and parathyroid hormone.
receptors on the same renal cells. On the other hand, cAMP formation in response to the direct adenylate cyclase stimulator forskolin remained unchanged in our study, indicating that the defect is located somewhere between the β-adrenergic receptors and adenylate cyclase, possibly at the level of G proteins. However, an alternative hypothesis would be that the increased renin release could result from increased sensitivity to submaximal effective agonist concentrations. The present study cannot rule out this possibility, and further studies will be necessary to test this hypothesis directly.

Taken together, the data of our study do not support the idea that increased renal adrenergic receptor numbers are accompanied by enhanced functional responsiveness. It should be noted, however, that functional responsiveness could theoretically occur via signaling pathways not investigated here, especially for α2-adrenergic receptors, but because such pathways have not been described in rat kidney, this alternate hypothesis remains purely speculative. Therefore, quantitative data on the effects of adrenergic agonists on renal vascular or tubular function or both are required to settle the question of whether renal adrenergic receptor responsiveness is altered in SHR and what pathophysiological importance that might have.

More generally, the question remains of why the number of α1- and β-adrenergic receptors increases in SHR kidney whereas the function apparently does not. Three explanations are possible: Function could decline to compensate for nonphysiological increases in number; number could increase to compensate for impaired function; or the two processes could be unrelated. Moreover, different explanations might exist for α1- and β-adrenergic receptors, because the former increase specifically in SHR, whereas the latter increase in many models of hypertension. Because reduced α1-adrenergic function was detected in SHR, in which receptor number remained unchanged, a compensatory reduction in α1-adrenergic responsiveness is unlikely. A direct test of the other possibilities awaits further experimental data, specifically, quantitation of the G protein subunits and effector enzymes.

In summary, we have demonstrated that α1-, α2-, and β-adrenergic receptor numbers are increased in SHR kidney and that these increases do not precede blood pressure elevations. Moreover, increased receptors are not accompanied by enhanced functional responsiveness or by an altered relative involvement of α1A- and α1B-adrenergic receptors in norepinephrine-stimulated inositol phosphate formation. Thus, our data provide further evidence that increased renal adrenergic receptor numbers are unlikely to contribute to the pathophysiology of blood pressure elevations in SHR.

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