On the Role of Renal \( \alpha \)-Adrenergic Receptors in Spontaneously Hypertensive Rats

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We tested the hypothesis that a genetically determined increase in renal \( \alpha \)-adrenergic receptor density might be a pathophysiologically important factor in the spontaneously hypertensive rat model of genetic hypertension. In a first study, we compared renal \( \alpha_1 \) and \( \alpha_2 \)-adrenergic receptor density with systolic blood pressure in 45 rats of an \( F_2 \) generation of Wistar-Kyoto × spontaneously hypertensive rat hybrids but were unable to detect significant cosegregation between either receptor density or blood pressure. In a second study, we determined renal \( \alpha_1 \) and \( \alpha_2 \)-adrenergic receptor density in Wistar-Kyoto and spontaneously hypertensive rat kidneys that were transplanted into an \( F_1 \) generation of Wistar-Kyoto × spontaneously hypertensive rat hybrids. Although Wistar-Kyoto kidneys lowered blood pressure in these animals and spontaneously hypertensive rat kidneys increased blood pressure, renal \( \alpha \)-adrenergic receptor densities were similar in membranes from both types of kidneys. Since rat kidney coexpresses \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors, we also investigated whether differential regulation of these two subtypes might conceal ongoing alterations. The \( \alpha_{1A}/\alpha_{1B} \)-adrenergic receptor ratio, however, was similar in Wistar-Kyoto rats, spontaneously hypertensive rats, and \( F_1 \) rats transplanted with a kidney from either strain. Taken together these data do not support the hypothesis that genetically determined alterations of renal \( \alpha \)-adrenergic receptor numbers play an important role in the development of elevated blood pressure in the spontaneously hypertensive rat. (Hypertension 1992;19:365–370)

Key Words: \( \alpha \)-adrenergic receptors • kidney • radioligand assay • essential hypertension • spontaneously hypertensive rats

Cross-transplantation studies have firmly established a role of the kidney in the development of elevated blood pressure in the spontaneously hypertensive rat (SHR) model of hypertension. Additional data suggest that this may also be the case for one or more subsets of patients with essential hypertension. Another consistent finding in the SHR and in many patients with essential hypertension is an altered function of the sympathoadrenal system. One reflection of this alteration is the repeated finding of an increased number of renal \( \alpha \)-adrenergic receptors in SHR; this increase precedes the elevation of blood pressure and is not found in models of acquired hypertension. Since some data link \( \alpha \)-adrenergic receptors to the control of renal sodium handling, we hypothesized that a genetically determined alteration of renal \( \alpha \)-adrenergic receptors may be an important pathophysiological component in genetic hypertension.

Alterations of renal \( \alpha \)-adrenergic receptors have been demonstrated for both major types, i.e., for \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors. Recent data demonstrate that each of these major types can be subdivided into at least three subtypes including \( \alpha_{1A}, \alpha_{1B}, \alpha_{1C}, \alpha_{2A}, \alpha_{2B} \), and \( \alpha_{2C} \).

The present study used two approaches to test our hypothesis that alterations of \( \alpha_1 \)- and/or \( \alpha_2 \)-adrenergic receptor subtypes play an important role in the development of elevated blood pressure in the SHR. First, we determined whether an increased renal \( \alpha \)-adrenergic receptor density might cosegregate with an increment in blood pressure in the \( F_2 \) generation of Wistar-Kyoto × spontaneously hypertensive rat (WKY × SHR) hybrids. Second, we determined whether hypertension induced by transplantation of an SHR kidney into the \( F_1 \) generation of WKY × SHR hybrids, when compared with transplantation of a Wistar-Kyoto (WKY) rat kidney, was accompanied by renal \( \alpha \)-adrenergic receptor alterations. Additionally, we investigated the balance of \( \alpha_{1A}/\alpha_{1B} \)-adrenergic receptors in SHR kidneys. The present data do not support the idea that alterations of renal \( \alpha \)-adrenergic receptors are a major determinant of blood pressure development in the SHR model of hypertension.

Methods

Animal Procedures

Animals for the present studies came from three sources: the \( F_2 \) generation of WKY × SHR hybrids were
generated in the breeding colony at the University of California, San Diego; WKY rats, SHR, and animals for the renal transplantation studies were from the colony at the University of Heidelberg; normotensive Wistar rats for the characterization of renal α₁-adrenergic receptor subtypes were from a commercial breeder (Lippische Versuchstierzucht, Extental, FRG). All animals were fed a standard laboratory chow that, according to the manufacturer, contained 0.2% NaCl and were given tap water ad libitum.

The F₂ generation of WKY×SHR hybrids was generated from inbred parental strains by crossing male SHR with female WKY rats; animals of the F₁ generation were then interbred to generate F₂ progeny. After determination of systolic blood pressure by a tail plethysmographic method at the age of 17 weeks, 45 female rats of the F₂ generation were killed.

Transplantation of WKY and SHR kidneys was performed as previously described. Briefly, kidneys from 16-week-old male WKY rats or SHR were transplanted to 16-week-old F₂ hybrids bred from WKY and SHR parents. During surgery for transplantation the recipients were unilaterally nephrectomized, and the second autotransplanted kidney was removed 1 week later. Recipients of WKY and SHR kidneys are designated WKY-TX and SHR-TX, respectively. All animals were killed 4 weeks after transplantation following determination of systolic blood pressure by a tail plethysmographic method. We have previously demonstrated that renal grafts from WKY rats and SHR do not significantly differ with regard to renal weight, renal blood flow, glomerular filtration rate, or plasma urea after transplantation.

Membrane Preparation and Binding Assays

Kidneys were decapsulated, rapidly frozen in liquid nitrogen, and stored at -70°C. Two methods were used to prepare renal membranes and perform the binding assays. For the study on the F₂ rats, crude renal membranes were prepared by homogenization in a Tissue-Mizer (Janke & Kunkel, Staufen, FRG) with subsequent centrifugation at 50,000g for 20 minutes as recently described. For all other experiments, kidneys were homogenized with a motor-driven glass/Teflon homogenizer (Braun, Melsungen, FRG) with a subsequent 1,000g spin followed by a 21,000g spin of the supernatant. The final pellets were resuspended in 50 mM TrisHCl, 0.5 mM EDTA at pH 7.5. The membranes were incubated in a total volume of 1 ml containing six different concentrations of [³H]prazosin (assay for α₁-adrenergic receptors) or 0.25 ml (assays for α₂-adrenergic receptors) containing six different concentrations of [³H]rauwolscine (F₂ study) or [³H]yohimbine (all other experiments). The incubations were performed for 60 minutes ([³H]rauwolscine binding) or 30 minutes (all other experiments) at 25°C and were terminated by rapid vacuum filtration over Whatman GF/C filters; an automatic cell harvester (Biomedical Research and Development Lab, Gaithersburg, Md.) was used in the F₂ experiments, whereas a manual vacuum filtration manifold was used in all other experiments. Nonspecific binding was defined as binding in the presence of 10 μM phentolamine. In competition binding experiments, 21 different concentrations of competitor were included in the assay. Protein content was determined by the method of Bradford using bovine immunoglobulin G as the standard. It should be noted that the membrane preparation used is rather crude, but we chose this method to avoid possible selective loss of receptors in one group during the preparation procedure.

Chemicals

Dye reagent for the protein assay was purchased from BioRad, Munich, FRG; oxymetazoline from Sigma, Munich, FRG; [³H]prazosin (specific activity = 80 Ci/mmol), [³H]yohimbine (specific activity = 80 Ci/mmol), and [³H]rauwolscine (specific activity = 80 Ci/mmol) were from New England Nuclear, Boston, Mass., or Dreieich, FRG. The following drugs were gifts of the respective companies: phentolamine (CIBA-GEIGY, Basel, Switzerland) and (+)-niguldipine and 5-methylurapidil (Byk Gulden, Konstanz, FRG).

Data Analysis

Saturation binding isotherms were analyzed by linear regression from Scatchard plots or by nonlinear regression analysis after fitting the data to a rectangular hyperbola; both methods yielded similar results. Competition binding experiments were analyzed by nonlinear regression with the iterative curve-fitting program INPLOT (GraphPAD Software, San Diego, Calif.); a two-site model was assumed if it resulted in a significant improvement of the goodness of fit (assessed by an F-test), otherwise a one-site model was assumed. The ratio of α₁A/α₁B-adrenergic receptors was determined from competition binding experiments with highly subtype-selective antagonists. Data are shown as mean±SEM of n experiments. Significances of differences among groups were analyzed by one-way analysis of variance followed by unpaired two-tailed t tests using Bonferroni corrections for multiple comparisons; statistical calculations were performed using the INSTAT program (GraphPAD Software), and a value of p<0.05 was considered to be significant.

Results

Studies in an F₂ Generation of WKY×SHR Hybrids

As expected, the systolic blood pressures in the F₂ generation of SHR×WKY hybrids varied considerably,
Receptor Subtypes

The competition curve for phentolamine was steep with female rats of an F2 generation of Wistar-Kyoto x spontaneously hypertensive rat hybrids. Each data point is the receptor density (Bmax) calculated from a saturation binding experiment using six concentrations of radioligand in duplicate.

Further studies to investigate possible differential regulation of α2-adrenergic receptor subtypes present in rat kidney. (+)-Niguldipine, 5-methyl-urapidil, and oxymetazoline yielded shallow biphasic competition curves that could be resolved into high- and low-affinity components (Figure 3, Table 1). Since (+)-niguldipine exhibited the greatest selectivity ratio, it was used in all further studies to investigate possible differential regulation of α2-adrenergic receptor subtypes.

Characterization of Renal α2-Adrenergic Receptor Subtypes

We used several compounds with known selectivity for α2-adrenergic receptor subtypes to characterize the subtypes present in rat kidney. (+)-Niguldipine, 5-methyl-urapidil, and oxymetazoline yielded shallow biphasic competition curves that could be resolved into high- and low-affinity components (Figure 3, Table 1). The competition curve for phenolamine was steep with a Hill slope not significantly different from unity (1.03 ± 0.18, n=4, Table 1). Since (+)-niguldipine exhibited the greatest selectivity ratio, it was used in all further studies to investigate possible differential regulation of α2-adrenergic receptor subtypes.

Transplantation Study in an F1 Generation of WKY x SHR Hybrids

In this part of our study, we compared renal α2-adrenergic receptors in WKY rats, SHR, and in an F1 generation of WKY x SHR hybrids in which a bilateral nephrectomy and a kidney transplantation from either a WKY rat or a SHR donor had been performed. The systolic blood pressure readings in the four groups were WKY < WKY-TX < SHR-TX < SHR (Figure 4). Thus, a WKY kidney lowers and a SHR kidney raises blood pressure in F1 hybrid recipients.

The density of α2-adrenergic receptors was not significantly different between renal membranes from WKY rats and SHR from the Heidelberg breeding colony (51.7 ± 3.7 versus 48.7 ± 3.7 fmol/mg protein) or between those from WKY-TX and SHR-TX (37.8 ± 2.8 versus 27.2 ± 4.6 fmol/mg protein; Figure 5). However, α2-adrenergic receptor density was significantly lower in WKY-TX compared with WKY and in SHR-TX compared with SHR (Figure 5). The affinities for [3H]prazosin did not differ significantly among groups (WKY, 0.31 ± 0.05 nM; SHR, 0.28 ± 0.03 nM; WKY-TX, 0.23 ± 0.02 nM; SHR-TX, 0.28 ± 0.03 nM). The α2/α1A-adrenergic receptor ratio did not significantly differ among all four groups (relative amount of α2/α1A-adrenergic receptors: WKY, 58 ± 3%; SHR, 67 ± 2%; WKY-TX, 63 ± 2%; SHR-TX, 62 ± 4%; Figure 6) and was similar to that observed in the prior experiments on renal membranes from normotensive Wistar rats (66±3%, Table 1).

Table 1. Drug Affinities at Rat Renal α2-Adrenergic Receptor Subtypes

<table>
<thead>
<tr>
<th>Drug</th>
<th>pKₐ</th>
<th>pKᵦ</th>
<th>% High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolamine</td>
<td>7.87±0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxymetazoline</td>
<td>7.55±0.19</td>
<td>5.91±0.17</td>
<td>31±7</td>
</tr>
<tr>
<td>5-Methyl-urapidil</td>
<td>8.90±0.14</td>
<td>7.02±0.05</td>
<td>50±1</td>
</tr>
<tr>
<td>(+)-Niguldipine</td>
<td>9.58±0.32</td>
<td>6.78±0.13</td>
<td>34±3</td>
</tr>
</tbody>
</table>

Data are mean±SEM of four experiments (n=3 for 5-methyl-urapidil) using 0.6 nM [3H]prazosin. Affinity constants are expressed as pKᵦ, i.e., −log of Kᵦ (the affinity constant). The competition curves for phenolamine were steep and could not be resolved into multiple components.
Renal α₂-adrenergic receptors in SHR (90.8±7.4 fmol/mg protein) were only slightly but not significantly higher than in WKY (82.5±10.6 fmol/mg protein, Figure 7). In F₁ hybrids transplanted with a WKY or SHR kidney, similar densities of renal α₂-adrenergic receptors were found that did not differ significantly (WKY-TX, 77.0±6.7 and SHR-TX, 83.5±17.3 fmol/mg protein, Figure 7). The affinity for the radioligand [³H]prazosin was similar in all four groups (WKY, 5.7±0.7 nM; SHR, 5.9±0.6 nM; WKY-TX, 5.1±0.4 nM; SHR-TX, 7.2±1.5 nM).

Discussion

The present data demonstrate that renal grafts of SHR donors can slightly reduce blood pressure to levels close to those of WKY rats in such recipients. These data confirm previous observations (for review, see Reference 1) from which it can be concluded that information expressed in the kidneys is predominantly if not exclusively responsible for the development of elevated blood pressure in the SHR model of genetic hypertension as well as in certain subsets of essential hypertensive patients.

We previously hypothesized that a genetically determined increase in renal α₂-adrenergic receptors might participate in the role of the kidney for the development of elevated blood pressure.² To link renal α₂-adrenergic receptors to the development of hypertension, it is important to identify the α₂-adrenergic receptor subtypes present in this tissue. The shallow biphasic competition curves for [³H]prazosin binding by (+)-niguldipine, 5-methyl-urapidil, and oxymetazoline in the present study demonstrate the presence of two types of α₂-adrenergic receptors in rat kidney. The high affinity

FIGURE 4. Bar graph shows systolic blood pressure in Wistar-Kyoto (WKY) rats (112±3 mm Hg), F₁ generation of WKY×spontaneously hypertensive rat (SHR) hybrids transplanted with a WKY kidney (WKY-TX, 122±5 mm Hg), F₁ generation of WKY×SHR hybrids with autochthonous kidneys (F₁, 139±3 mm Hg), F₂ generation of WKY×SHR hybrids transplanted with a SHR kidney (SHR-TX, 161±5 mm Hg), and SHR (186±5 mm Hg). Each bar is mean±SEM of seven to 13 animals.

FIGURE 5. Scatter plots show renal α₂-adrenergic receptor density (Bₐα₂) in Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and the F₁ generation of WKY×SHR hybrids transplanted with either a WKY or SHR kidney (WKY-TX and SHR-TX, respectively). ○, Receptor density (Bₐα₂) in a single animal; ●, mean±SEM of all animals in the group. *p<0.05 and **p<0.01 vs. the receptor density in the respective nontransplanted kidney.

FIGURE 6. Scatter plots show ratio of α₁α₂-adrenergic receptors expressed as percentage of α₁α₂-adrenergic receptors in Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and the F₁ generation of WKY×SHR hybrids transplanted with either a WKY or SHR kidney (WKY-TX and SHR-TX, respectively). ○, Receptor density (Bₐα₂) in a single animal; ●, mean±SEM of all animals in the group.

FIGURE 7. Scatter plots show renal α₁-adrenergic receptor density (Bₐα₁) in Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and the F₁ generation of WKY×SHR hybrids transplanted with either a WKY or SHR kidney (WKY-TX and SHR-TX, respectively). ○, Receptor density (Bₐα₁) in a single animal; ●, mean±SEM of all animals in the group.
component for all three drugs corresponds to \( \alpha_{1A} \)-adrenergic receptors, whereas the low affinity component identifies \( \alpha_{1D} \)-adrenergic receptors.\textsuperscript{12,13} From our data an \( \alpha_{1A}/\alpha_{1D} \)-adrenergic receptor ratio of 40:60 can be calculated. Our data have been obtained by other investigators using isolated renal cells or renal homogenates.\textsuperscript{14,15} Previous studies have demonstrated that the \( \alpha_{1D} \)-adrenergic receptors in rat kidney are predominantly, if not exclusively, of the \( \alpha_{1D} \)-subtype.\textsuperscript{9,16-18} Thus, pharmacological means can detect three types of \( \alpha \)-adrenergic receptors in rat kidney, \( \alpha_{1A} \), \( \alpha_{1D} \), and \( \alpha_{2} \)-adrenergic receptors. Northern blotting has also confirmed the presence of these three \( \alpha \)-adrenergic receptor subtypes in rat kidney.\textsuperscript{5,6,19} The existence of \( \alpha_{2A} \)-adrenergic receptors in rat kidney remains unclear since it is detected by Northern blot analysis\textsuperscript{5} but not by radioligand binding.\textsuperscript{9,18}

As reviewed previously,\textsuperscript{2,4} various laboratories have presented data indicating that an elevation of renal \( \alpha \)-adrenergic receptors may precede blood pressure increases in SHR but may be absent in animal models of acquired hypertension. The present study sought to examine whether renal \( \alpha \)-adrenergic receptors cosegregate with blood pressure in the \( F_{2} \) progeny of WKYxSHR hybrids. However, our data fail to detect a significant correlation between either renal \( \alpha_{1} \)- or \( \alpha_{2} \)-adrenergic receptor density and systolic blood pressure in such animals. Since hypertension in the SHR is likely to be a multigenic disease, the possibility remained that an important contribution of \( \alpha \)-adrenergic receptors had been overlooked in the crossbreeding studies due to the variation in our measurements in relation to the number of animals studied. Therefore, we undertook studies in renally transplanted animals as a second means to investigate this question.

For several reasons, our data argue against an important role of renal \( \alpha_{1A} \), \( \alpha_{1D} \), or \( \alpha_{2} \)-adrenergic receptor number for the development of genetic hypertension in SHR: First, SHR and WKY rats from the Heidelberg breeding colony were clearly distinguished by blood pressure but did not exhibit significantly different amounts of \( \alpha_{1} \)- or \( \alpha_{2} \)-adrenergic receptors. On the other hand, we detect significant increases in renal \( \alpha_{1} \)- and \( \alpha_{2} \)-adrenergic receptors compared to WKY rats and SHR from a different breeder (Möller, Denmark) with the same methods (Reference 20 and data not shown). Previous studies have uniformly detected elevated renal \( \alpha_{2} \)-adrenergic receptors in SHR, but increases in renal \( \alpha_{1} \)-adrenergic receptors were found in some studies and not in others (as reviewed in References 2 and 4). The fact that SHR from various breeders are clearly hypertensive but only some exhibit increased \( \alpha_{1} \)- or \( \alpha_{2} \)-adrenergic receptors argues against an important role for such changes. Second, SHR kidneys that can induce hypertension in immunotolerant recipient do not have significantly different \( \alpha_{1} \)- or \( \alpha_{2} \)-adrenergic receptors from those of WKY kidneys that lowered blood pressure. We cannot exclude that denervation affected the receptor numbers in the transplanted kidneys, but we do not consider the development of denervation supersensitivity very likely in this case since the number of \( \alpha_{2} \)-adrenergic receptors in the transplanted kidneys was very similar to those in the donors and the number of \( \alpha_{1} \)-adrenergic receptors was even lower in the transplanted kidneys versus the respective donors. Moreover, the effects of denervation, if any, should be similar for grafts from WKY rats and SHR. Third, we have excluded that a possible redistribution of \( \alpha_{1A} \)-adrenergic receptor subtypes conceals \( \alpha_{1D} \)-adrenergic receptor changes since the ratio of \( \alpha_{1A}/\alpha_{1D} \)-adrenergic receptors did not differ significantly among groups. Taken together with the data from the \( F_{2} \) generation of WKYxSHR hybrids, these data do not support the thesis that a genetically determined increase in renal \( \alpha_{1} \)- or \( \alpha_{2} \)-adrenergic receptors is crucial for the development of hypertension in the SHR model. On the other hand, these data do not allow conclusions regarding the possible relevance of alterations in \( \alpha \)-adrenergic receptor signaling in hypertension.

Recently reported preliminary data suggest that the gene for the \( \alpha_{2A} \)-adrenergic receptor exhibits restriction fragment length polymorphism between WKY rats and SHR\textsuperscript{21} and that an \( F_{2} \) generation of WKYxSHR hybrid rats with a homozygous SHR genotype at the \( \alpha_{2A} \)-locus had significantly higher blood pressure than rats with a homozygous WKY genotype, whereas rats with a heterozygous genotype had intermediate blood pressure.\textsuperscript{22} These data suggest that the gene for the \( \alpha_{2A} \)-adrenergic receptor or a very closely related gene may play an important role in the development of elevated blood pressure in the SHR. As stated above, \( \alpha_{2A} \)-adrenergic receptors are detected in rat kidney by Northern blotting but cannot be detected by radioligand binding. Thus, it remains unclear how these data relate to the hypothesis of elevated renal \( \alpha_{1} \)-adrenergic receptors as an important factor in the pathophysiology of hypertension in the SHR. They may, however, be relevant to our understanding of the pathophysiology of human essential hypertension since human kidneys express predominantly \( \alpha_{2A} \)-adrenergic receptors (80% or more of total \( \alpha_{2} \)-adrenergic receptors\textsuperscript{18}), and a restriction fragment length polymorphism for the \( \alpha_{2A} \)-adrenergic receptor gene has also been demonstrated in humans.\textsuperscript{23} Whether polymorphism for the \( \alpha_{2A} \)-adrenergic receptor gene is associated with a family history of human essential hypertension is unknown. It should be mentioned, however, that patients with a family history of hypertension exhibit higher platelet \( \alpha_{2A} \)-adrenergic receptor densities than those without such a family history.\textsuperscript{24}

In summary, our data provide evidence that possible alterations of renal \( \alpha_{1A} \), \( \alpha_{1D} \), or \( \alpha_{2} \)-adrenergic receptor number do not play a major pathophysiological role in the development of genetic hypertension in the SHR. Alterations of \( \alpha_{2A} \)-adrenergic receptors, however, may be important in the SHR and may also play a role in human hypertension. Further studies are needed to establish the role of possible alterations of \( \alpha \)-adrenergic receptor function in hypertension.

Note added in proof. Using the novel radioligand \( [\text{H}]\text{RX821002}, detection of \alpha_{2A} \)-adrenergic receptors in rat kidney has recently become feasible.\textsuperscript{25}

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