Renal \( a \)-Adrenergic Receptor Response Coupling in Spontaneously Hypertensive Rats

WILLIAM B. JEFFRIES, ELSA YANG, AND WILLIAM A. PETTINGER

SUMMARY Renal sympathetic antidiuretic, antinatriuretic, and vasoconstrictor responses are mediated by \( a \)-adrenergic receptors in the normal rat. Since the renal nerve has been implicated in the pathogenesis of rat genetic hypertension, we investigated renal \( \alpha \)-adrenergic receptor coupling to phosphoinositide turnover in spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY). In cortical slices from adult (13-week-old) SHR and WKY, stimulation with norepinephrine (10\(^{-9}\) M) caused a concentration-dependent increase in accumulation of \(^{3}\)H]inositol phosphates. However, dose-response curves for SHR characteristically displayed a depression of the maximum response as compared with those for WKY. Baseline accumulation of \(^{3}\)H]inositol phosphates was not different between strains (39.4 ± 2.2 cpm/mg tissue/hr for WKY and 34.4 ± 2.1 cpm/mg tissue/hr for SHR slices; \( n = 5 \) rats/group, determined in triplicate). Antagonist competition studies revealed that norepinephrine-stimulated (10\(^{-9}\) M) \(^{3}\)H]inositol phosphate accumulation was mediated by \( a \)-adrenergic receptors (IC\(_{50}\) for prazosin: 65 ± 11 nM for SHR and 64 ± 5 nM for WKY). The reduction in norepinephrine-stimulated \(^{3}\)H]inositol phosphate accumulation in SHR cortex was not the result of hypertension, since it was also present in cortical slices from young (4-week-old) SHR in which the blood pressure was not yet significantly different from that in WKY and since \(^{3}\)H]inositol phosphate accumulation was unchanged from control values in rats made hypertensive by treatment with deoxycorticosterone acetate. Scatchard analysis of \(^{3}\)H]prazosin binding in renal cortical membranes of young and adult SHR and WKY revealed no significant differences in \( a \)-adrenergic receptor density or affinity between strains at either age. Our results suggest that renal \( a \)-adrenergic receptor coupling to phospholipase C is less efficient in SHR than in WKY. This unpaired response is not the result of hypertension or changes in receptor density; this defect may play a role in increased renal sympathetic nerve activity and in the development or maintenance of hypertension in SHR. (Hypertension 12: 80-88, 1988)

KEY WORDS • inositol phosphates • phospholipase C • kidney slices • prazosin • norepinephrine

The renal sympathetic nerve plays an important role in renal tubular electrolyte reabsorption and renovascular tone.\(^1\) Low frequency stimulation of the rat renal nerve causes an \( a \)-adrenergic receptor-mediated increase in tubular sodium and water reabsorption.\(^2\) Higher frequency renal nerve stimulation results in renovascular constriction mediated by \( a \)-adrenergic receptors.\(^3\) In light of the influence of the sympathetic nervous system on sodium and water reabsorption and renovascular tone, the potential importance of the renal nerve in the pathogenesis of experimental hypertension is well recognized.\(^4\) Particularly compelling is evidence that efferent renal nerve activity is elevated and junctional norepinephrine release is enhanced\(^5\) in spontaneously hypertensive rats (SHR) when compared with Wistar-Kyoto rats (WKY)\(^6\) and that renal denervation delays the onset of genetic hypertension.\(^8\) Very recent evidence suggests that neuronal reflexes are impaired in SHR kidneys.\(^9\) Since \( a \)-adrenergic receptors mediate important functions of the renal nerve, we hypothesize that this adrenergic receptor subtype plays an integral role in the pathogenesis of hypertension in SHR. Interestingly, it was recently reported\(^10\) that renal membrane \( a \)-adrenergic receptor density is increased in SHR compared with WKY.

These findings suggest that \( a \)-adrenergic receptor regulation is defective in SHR, since the receptors fail to down-regulate (and, in fact, are increased in density) under conditions of increased sympathetic nerve activity (and hence, increased junctional norepinephrine concentration or turnover, or both). It has thus been proposed\(^11\) that this defect is responsible for enhanced sodium and water reabsorption and increased renovascular tone in SHR.
a,-Adrenergic receptor-mediated signals are transduced through an interaction with phospholipase C, which then hydrolyzes phosphoinositides and polyphosphoinositides to form inositol phosphates and diacylglycerol. Thus, the rate of formation of these products can be used to assess the relative efficiency of a,-adrenergic receptor coupling to phospholipase C.

In the present study, we established a kidney slice preparation to examine norepinephrine-stimulated accumulation of inositol phosphates. With this model we tested the hypothesis that altered renal a,-adrenergic receptor regulation in SHR causes an increase in phosphoinositide turnover and hence an increase in a,-adrenergic receptor-mediated effects in the kidney. The results of our study indicate that a,-adrenergic receptor coupling efficiency is reduced, rather than increased, in the kidneys of adult and weanling SHR compared with those of WKY.

Materials and Methods

Animals

Adult (weight, 250-300 g) male Sprague-Dawley rats (Harlan, Houston, TX, USA) were used to establish the kidney slice model. For experiments with genetically hypertensive rats, male SHR or WKY (Harlan) were purchased at an age of 3 or 12 weeks and were housed for 1 week in our animal room before use. The rats were allowed free access to standard laboratory chow and tap water. For studies in deoxycorticosterone acetate (DOCA)-sodium hypertensive rats, male Sprague-Dawley rats were purchased as described and divided into two groups. One group received 5-mg injections of DOCA twice weekly for 3 weeks with a 1% NaCl reduced to 108 mM to inhibit inositol-1-phosphatase and myo-inositol (30 µM) to prevent any subsequent relabeling of phosphatidylinositol. In some experiments, prazosin (10^-5-10^-3 M) or rauwolscine (10^-5 to 3 x 10^-7 M) was present in the incubation buffer at this point. Norepinephrine (10^-3-10^-7 M, each concentration in triplicate for each kidney) was then added after 10 minutes and incubated for an additional 20 minutes. The incubations were terminated by removing each slice from the buffer and placing it into 1 ml of an extraction solution containing chloroform, methanol, and HCl (0.01 N) in a ratio of 0.5 : 1.0 : 0.4 (vol/vol/vol).

Extraction of Inositol Phosphates

The tissue was homogenized with a Brinkman polytron (7-mm generator, Westbury, NY, USA). The polytron was washed after homogenization of each sample with 0.9 ml of the extraction mixture, and the two fractions were pooled and centrifuged at 150 g for 10 minutes. The resulting supernatant was added to 1 ml of a 1 : 1 (vol/vol) mixture of chloroform and 0.01 N HCl to separate the phases. Total inositol phosphates were then determined using a modification of the method of Barridge et al. Briefly, the aqueous phase from each sample was separated and placed onto a column containing 1.5 ml of Dowex AGI-X8 anion exchange resin (200-400 mesh, formate form). [3H]inositol was then removed by washing with 10 ml of a 5 mM myoinositol solution. The glycerol inositol phosphate fraction was then eluted with 5 ml of a 4.0 mM sodium tetraborate/60 mM sodium formate solution. Total inositol phosphates were then eluted with 5 ml of a formic acid/ammonium formate solution (0.1 : 1.2 M, respectively). A 0.1 ml aliquot of the eluent was then pipetted into scintillation vials containing 10 ml of Triton/toluene scintillation cocktail.

To ensure adequate incorporation of [3H]inositol into membrane phospholipids, a 0.5 ml aliquot of the organic phase of each sample was added to 10
ml of scintillation cocktail in scintillation vials. All samples were then counted for 10 minutes. Preliminary studies using thin-layer chromatography established that more than 95% of the organic phase counts originated from $[^3H]$phosphatidylinositol.

Radioligand Binding Assays

Rats of each strain and age group were killed, and the decapsulated kidneys were flash-frozen (dry ice-methanol) and stored at — 40 °C for subsequent radioligand binding assays. The frozen kidneys were thawed at 4 °C, and cortical tissue was dissected free of medulla. In some experiments, renal cortical slices were prepared and incubated in K-H buffer as already described ($[^3H]$mipo-inositol omitted) and the slices were pooled and used fresh in the preparation of renal membranes. Cortical plasma membranes were prepared and binding studies performed as described by Williams et al. with modifications by Schmitz et al. Renal cortical membranes were first washed with 5 mM EDTA in cold 50 mM Tris buffer (pH 7.5) and then incubated with radioligand in 50 mM Tris buffer and 5 mM MgCl at 25 °C for 30 minutes. $[^3H]$Prazosin (0.1-4.0 mM) was used to construct a Scatchard plot in duplicate for each assay. The maximal concentration of ligand was at least five times the $K_d$ to ensure saturation of receptor sites. Incubation was terminated by instantaneous filtration through Whatman GF/C glass filters (Clifton, NJ, USA). The filters were washed with three 5-ml aliquots of cold buffer, dried, and placed in scintillation vials for counting. Specific binding was defined with 10 μM phentolamine. Agonist displacement studies were also performed with norepinephrine (10–6–10–3 M). The $[^3H]$prazosin concentration in these studies was approximately 3 nM. Differential elution of inositol phosphates with a 50% effective concentration (EC50) was performed as described by Williams et al.

Drugs Used

The drugs used in this study and their sources are as follows: norepinephrine HCl, arginine vasopressin, angiotensin II, 5-hydroxytryptamine HCl, carbachol (Sigma Chemical, St. Louis, MO, USA); prazosin HCl (Pfizer, New York, NY, USA); rauwolscine HCl (Dr. Carl Roth, Karlsruhe, West Germany); $[^3H]$prazosin, 81 μCi/mmol (New England Nuclear).

Statistics

Radioligand binding and displacement data were analyzed with the curve-fitting program LIGAND. In other analyses, homogeneity of variances was established with Bartlett’s test. The differences between groups were determined with the Newman-Keuls multiple comparison test.

Results

Blood pressure was not different between SHR and WKY at 4 weeks of age (110 ± 9 and 113 ± 9 mm Hg, respectively, $n = 5$ /group) but was significantly higher in adult (13 weeks) SHR than in adult WKY (172 ± 5 vs 121 ± 4 mm Hg; $p < 0.001$). Rats treated with a DOCA-sodium regimen were hypertensive with respect to olive oil-tap water controls at the time of death (systolic pressure, 142.2 ± 6.4 vs 125.7 ± 5.8 mm Hg; $p < 0.001$).

Validation of the Kidney Slice Model

Slices derived from adult SHR and WKY did not differ with respect to mean wet weight (10.6 ± 0.2 vs 10.5 ± 0.3 mg, respectively; $n = 90$ slices/group), protein content (50 ± 5 vs 50 ± 5 mg wet tissue), or water content (78.35 ± 0.24 vs 78.99 ± 0.23%). In the experiments comparing DOCA-sodium hypertensive rats with controls, no differences were observed between groups in mean slice wet weight (11.2 ± 0.3 vs 10.9 ± 0.3 mg, respectively) or protein content (99 ± 12 vs 92 ± 12 μg/g wet weight).

Preliminary studies in Sprague-Dawley rats were used to determine the appropriate experimental parameters for subsequent studies. These studies revealed that accumulation of tritium in the slices was linear with time (0-120 minutes). Norepinephrine (10–6 M) added after the labeling period caused an increase in $[^3H]$inositol phosphates that was linear with time, reaching a maximum at 20 minutes (Figure 1). The 20-minute incubation period was used for subsequent studies. Norepinephrine caused a dose-related rise in accumulation of inositol phosphates with a 50% effective concentration (EC50), of approximately 3 nM. Differential elution of inositol monophosphate, inositol bisphosphate, and inositol trisphosphate by the method of Berridge et al. revealed that more than 90% of the labeled species eluted was in the form of inositol monophosphate. Norepinephrine (10–6 M) stimulation of inositol phosphate accumulation was greatly reduced when performed in a buffer free of LiCl (control = 30.1 ± 4.1 cpm/mg slice; norepinephrine-stimulated = 42.2 ± 2.6 cpm/mg slice).

Inositol Phosphate Accumulation in Genetically Hypertensive Rats

Figure 2 shows the dose response to norepinephrine for total $[^3H]$inositol phosphate accumulation in adult SHR and WKY. The baseline (nonstimulated) labeling of $[^3H]$inositol phosphates (see Figure 2) was not different between strains. However, norepinephrine-stimulated accumulation of $[^3H]$inositol phosphates was consistently higher at each concentration of NE in WKY slices, compared with SHR slices (see Figure 2). The EC50 values for norepinephrine were not different between strains (5.0 ± 1.0 μM for SHR and 3.4 ± 0.7 μM for WKY). To investigate whether this phenomenon was the result of the higher blood pressure of adult SHR, we repeated the studies in slices from 4-week-old rats. Figure 3 demonstrates that, although the norepinephrine-stimulated accumulation of $[^3H]$inositol phosphates was reduced relative to
REDUCED a.-ADRENERGIC RECEPTOR COUPLING IN SUR/Jeffries et al.

FIGURE 1. Characteristics of norepinephrine (NE)-stimulated accumulation of [3H]inositol phosphates in renal cortical slices of Sprague-Dawley rats. A. Time course for NE-stimulated accumulation of inositol phosphates. Cortical slices from Sprague-Dawley rats were incubated in Krebs-Henseleit buffer (37 °C) with 3 μCi [3H]myo-inositol for 60 minutes. The slices were then transferred individually to tubes containing Krebs-Henseleit buffer with 10 mM LiCl (isotonic) and 30 μM cold myo-inositol for 10 minutes. NE (10^{-5} M) or buffer (basal time controls) was then added and incubated for the times shown. Incubations were terminated by placing the slices in (0.4:1:0.5) 0.1 N HCl:methanol:chloroform. Total labeled inositol phosphates were extracted and quantified as described in Materials and Methods. Each point represents the mean response in cortical slices from three rats, determined in triplicate. B. Dose-response curve for NE. Slices were prepared as just described and were incubated with various concentrations of NE for 20 minutes.

FIGURE 2. Norepinephrine (NE)-stimulated accumulation of inositol phosphates (IPs) in renal cortical slices from adult SHR and WKY. Slices were preincubated with 3 μCi [3H]myo-inositol for 60 minutes, then transferred individually to tubes containing Krebs-Henseleit buffer with 10 mM LiCl (isotonic) and 30 μM cold myo-inositol for 10 minutes. NE (10^{-4} M) was added (three slices/dose/strain), and incubations proceeded for 20 minutes. Basal values represent slices incubated in the absence of NE. Total labeled IPs were extracted and quantified as described in Materials and Methods. Each point represents the pooled results for 15 slices (triplicate determination in each of five animals) except for the 10^{-3} M concentration, which represents two rats/strain (six slices). Single (p < 0.05), double (p < 0.01), and triple asterisks (p < 0.001) indicate significant difference between groups.

To further investigate the possibility that reduced norepinephrine-stimulated phospholipase C activity was a result of hypertension, we performed experiments with kidney slices obtained from DOCA-sodium hypertensive and normotensive control rats. Figure 4 shows that there was no significant differ--
ence in norepinephrine-stimulated inositol phosphate accumulation between DOCA-sodium and control rats, suggesting that decreased adrenergic receptor-stimulated phosphoinositide turnover is not a response to elevated blood pressure and is a genetic characteristic of the SHR strain.

The norepinephrine-stimulated accumulation of [3H]-inositol phosphates was mediated by α₁-adrenergic receptors, since the maximal response to norepinephrine was potently inhibited by the selective α₁ adrenergic receptor antagonist prazosin (Figure 5). The selective α₂-adrenergic receptor antagonist rauwolscine was approximately 1000 times less potent than prazosin. The IC₅₀ values of the antagonists were not different between strains.

To determine whether this difference in α₁ adrenergic receptor-mediated inositol phosphate accumulation was a reflection of a generalized phospholipase C deficiency, we examined other known activators of phospholipase C in SHR and WKY kidney slices (Figure 6). Norepinephrine was the most potent activator of phospholipase C in cortical slices, followed by (in descending order) angiotensin II, serotonin, histamine, carbachol, and vasopressin. Agonist-stimulated inositol phosphate accumulation was significantly different (p < 0.001) between strains only for norepinephrine. Thus, the altered efficiency of inositol phosphate accumulation is not likely to result from a strain difference in basal phospholipase C activity but is related specifically to α₁-adrenergic receptor-coupled phospholipase C activity.

α₁-Adrenergic Receptor Binding Studies

Saturation studies with [3H]prazosin yielded linear Scatchard plots (Figure 7 and Table 1). No significant difference was found between SHR and WKY.
WKY cortical \( \alpha_1 \)-adrenergic receptor binding characteristics at either age. We also examined norepinephrine displacement of \([\text{H}]\)prazosin in renal cortex from adult SHR and WKY and again found the curves to be identical between strains at both ages (Figure 8; see Table 1). One published study\(^{18}\) reported that the density of \([\text{H}]\)prazosin binding sites in SHR kidneys was approximately double that of WKY kidneys. We therefore performed binding assays with \([\text{H}]\)prazosin using the conditions (Na-K-PO\(_4\) buffer, no EDTA wash) used by these authors. In the Na-K-PO\(_4\) buffer the apparent receptor density was slightly higher (25\%) in SHR (maximum binding capacity = 84.2 ± 6.1 fmol/mg protein; \( n = 6 \)) than in WKY (maximum binding capacity = 68.4 ± 3.5 fmol/mg protein; \( n = 5 \)), and the difference was just below the level of statistical significance (\( p < 0.06 \)). No significant difference between strains was observed in the \( K_d \) (0.87 ± 0.18 nM for SHR and 0.73 ± 0.17 nM for WKY) for \([\text{H}]\)prazosin.

Thus, we observed that the buffer composition affected the apparent maximum binding capacity for prazosin binding. The density of receptors could have been altered disproportionately between strains during the course of our incubations in K-H buffer in our functional assays. Such a change could account for the interstrain differences in inositol phosphate accumulation. Therefore, we performed \( \alpha_1 \)-adrenergic receptor binding assays in membranes prepared from cortical slices preincubated in K-H buffer. We again found no difference in maximum binding capacity (SHR = 104.4 ± 5.9 fmol/mg protein; WKY = 108 ± 9.6 fmol/mg protein) or \( K_d \) (SHR = 0.61 ± 0.1 nM; WKY = 0.60 ± 0.19 nM) between strains (\( n = \) three rats/group). Inclusion of norepinephrine (10\(^{-4}\) M) in the preincubation medium reduced binding density by 12.6 ± 4.0\% in SHR and by 13.7 ± 3.9\% in WKY, with no change in affinity.

**Discussion**

\( \alpha_1 \)-Adrenergic receptor–mediated signal transduction is thought to occur through interaction with a G protein and the phosphodiesterase phospholipase C.\(^{19}\) Activation of phospholipase C results in the hydrolysis of membrane phosphoinositides and polyphosphoinositides to yield the putative second messengers diacylglycerol and inositol 1,4,5-trisphosphate.\(^{20}\) In the present study, we have quantified the accumulation of total inositol phosphates in response to norepinephrine in kidney slices of SHR and WKY. The results indicate that renal \( \alpha_1 \)-adrenergic receptors are less efficiently coupled to phospholipase C in SHR than in WKY. This difference preceded the development of marked hypertension in these animals and was not present in DOCA-sodium hypertensive rats, suggesting that this abnormality was not the result of elevated blood pressure in SHR. The reduced efficiency of renal phospholipase C in SHR was selective for \( \alpha_1 \)-adrenergic receptor stimulation since the response to other agonists was similar between strains.

Norepinephrine-stimulated inositol phosphate accumulation was reduced in SHR despite a similar renal \( \alpha_1 \)-adrenergic receptor density in SHR compared with WKY. Thus, this interstrain variation apparently does not emanate from a difference in receptor capacity or affinity. It has been proposed\(^{18}\) that increases in renal \( \alpha_1 \)-adrenergic receptor density may amplify postjunctional responses in SHR, contributing to, or causing, hypertension in this strain. However, we have found
that, although the number of renal α,-adrenergic receptors in SHR is either not different (Tris buffer) or slightly higher (phosphate buffer) than that in WKY, the relative ability of SHR α,-adrenergic receptors to transduce signals is reduced when compared with that of WKY, not increased. Thus, changes in α,-adrenergic receptor binding capacity, taken alone, cannot reliably predict altered signal transduction processes.

α,-Adrenergic receptor-phospholipase C coupling has been the subject of a limited number of investigations in genetically hypertensive rats. Table 2 summarizes some of the recent studies. In brain tissue of SHR, α,-adrenergic receptor density is increased compared with WKY, 29-30 and Feldstein et al. 29 have shown that α,-adrenergic receptor stimulation of inositol phosphate accumulation was decreased in neuronal cell cultures harvested from 1-day-old SHR. Our results from an entirely different model system appear to be quite similar to those of Feldstein et al., 29 suggesting a general distribution of this functional abnormality in α,-adrenergic receptor coupling in SHR. However, results from vascular tissue have been conflicting. Heagerty et al. 32 have shown that α,-adrenergic receptor-stimulated production of [3H]inositol phosphates was reduced in the aorta of adult SHR compared with WKY, with no change in the basal activity, similar to our findings in the kidney. However, Uehara et al. 33 reported that basal phospholipase C activity was significantly enhanced compared with WKY in neuronal cell cultures harvested from 15-week-old SHR. These findings are in contrast to those of Kawaguchi et al. 29 who showed that basal phospholipase C activity differs between the strains. 33 In the present study, the response characteristics of normotensive Sprague-Dawley rats closely resemble those of WKY (compare Figures 3 and 5), indicating an abnormality of SHR rather than of WKY.

The mechanism for the reduction of renal α,-adrenergic receptor-phospholipase C coupling efficiency in SHR is unclear. The data suggest that the site of the alteration is at the level of the α,-adrenergic receptor-phospholipase C interaction, since basal and nonadrenergic-stimulated inositol phosphate accumulation was similar between strains. In the present study, we were unable to identify any differences in α,-adrenergic receptor binding characteristics between strains that would suggest differences in the α,-adrenergic receptor protein itself. SHR renal α,-adrenergic receptors may be desensitized from exposure to a higher level of renal sympathetic nerve stimulation 29 without a loss in receptor number. However, α,-adrenergic receptor density was increased and coupling efficiency to phospholipase C was decreased compared with WKY in neuronal cell cultures harvested from neonatal SHR, implying that this phenomenon is independent of tonic agonist stimulation. The combined evidence suggests a fundamental abnormality in α,-adrenergic receptor coupling in the SHR.

α,-Adrenergic receptors are under the regulatory influence of protein kinase C. Stimulation of α,-adrenergic receptors causes activation of phospholipase C and hydrolysis of phosphoinositides, yield-

---

**TABLE 2. α,-Adrenergic Receptor Binding Capacity and Phospholipase C Activity in SHR and WKY**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>α,-Adrenergic receptor density</th>
<th>Basal</th>
<th>α,-Adrenergic receptor-stimulated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Unchanged</td>
<td>Unchanged</td>
<td>Decreased</td>
<td>Present study</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>ND</td>
<td>Sanchez et al. 27</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>ND</td>
<td>Graham et al. 32</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>ND</td>
<td>Kawaguchi et al. 29</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>Increased*</td>
<td>ND</td>
<td>Feldstein et al. 29</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>ND</td>
<td>Pullen et al. 30</td>
</tr>
<tr>
<td>Brain</td>
<td>Increased</td>
<td>Unchanged</td>
<td>Decreased</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>Increased</td>
<td>ND</td>
<td>Increased!</td>
<td>Kobayashi et al. 31</td>
</tr>
<tr>
<td></td>
<td>Unchanged</td>
<td>ND</td>
<td>Increased!</td>
<td>Heagerty et al. 32</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>Unchanged*</td>
<td>Sharma et al. 34</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>Decreased</td>
<td>Sharma et al. 34</td>
</tr>
<tr>
<td></td>
<td>Increased</td>
<td>ND</td>
<td>Unchanged</td>
<td>Uehara et al. 31</td>
</tr>
</tbody>
</table>

Table represents changes in parameter values for SHR relative to those for WKY. ND = not determined.

*Stroke-prone SHR.
†Five-week-old rats.
‡Fifteen-week-old rats.
Diacylglycerol and inositol phosphates. Activation of protein kinase C with tumor-promoting phorbol esters causes an uncoupling of hepatic, hippocampal, and vascular α1 adrenergic receptors from associated G proteins. This uncoupling is accompanied by a loss of high affinity agonist binding and phosphorylation of the receptor protein. It is tempting to hypothesize that the reduced level of α1-adrenergic receptor coupling in SHR is due to enhanced protein kinase C activity, which could result in a greater proportion of receptors in the phosphorylated (inactive) state. Interestingly, two reports have described an increase in protein kinase C activity in vascular tissue and platelets from SHR compared with WKY.

Excessive renal retention of sodium and water has been suggested as a cause of experimental and human essential hypertension (see Reference 44 for review). The renal nerve may have an important role in the pathogenesis of hypertension in SHR since chronic bilateral denervation of the kidneys in young SHR delays the onset of hypertension until reinnervation is established. Acute renal denervation produces a diuresis and natriuresis in young SHR but not in WKY, demonstrating an increased dependence on tonic renal nerve activity for sodium and water handling in SHR. Responses to renal nerve stimulation in SHR and WKY are mediated solely by α1-adrenergic receptors. Efferent renal sympathetic nerve activity is elevated in SHR compared with WKY, and norepinephrine release is augmented in kidneys of SHR.

An attractive hypothesis regarding the pathogenesis of rat genetic hypertension has been that the renal sympathetic responses may be enhanced in SHR, leading to excess sodium and water retention and increased vascular tone. However, our results suggest that renal sympathetic signal transduction beyond the α1-adrenergic receptor is impaired in SHR. If renal postjunctional responses are enhanced in SHR, the site in the sequence must be beyond the activation of phospholipase C.

Our results and those of Feldstein et al.25 raise the interesting possibility that a biochemical defect in α1-adrenergic receptor coupling may be the cause of enhanced renal sympathetic activity in SHR. The reduced coupling could result in a reduction in afferent neuronal feedback to the brain, which could in turn cause an increase in efferent sympathetic nerve activity. We have recently noted similarities between SHR and a model of impaired α1 adrenergic receptor function, the Sprague-Dawley rat treated chronically with prazosin. Chronic α1-adrenergic receptor blockade causes activation of the sympathetic nervous system, with increased renal α2-adrenergic receptor density, augmented renal neuronal uptake of norepinephrine, and enhanced activity of prejunctional α2-adrenergic receptors. Thus, this model of impaired α1-adrenergic receptor function resembles the SHR in several respects. It is presently unknown whether deficient α1-adrenergic receptor-phospholipase C signal transduction drives the changes in sympathetic activity just described. Interestingly, Kopp et al. recently reported that renal sensory feedback was impaired in SHR compared with WKY.

An additional point of interest is the relationship between renal α1-adrenergic and α2-adrenergic receptor populations. Chronic blockade of α1-adrenergic receptors increases the density of renal α2-adrenergic receptors, similar to that seen in SHR. It is possible to speculate from these data that renal α2-adrenergic receptors somehow regulate α2-adrenergic receptor density, since impairment of α1-adrenergic receptor function causes increased α2-adrenergic receptor density. If this hypothesis is true, then abnormal renal α2-adrenergic receptor expression in hypertensive rats might result from a defect in α1-adrenergic receptor signal transduction. This sequence of events may explain the apparent lack of functional importance of high α2-adrenergic receptor density in the SHR kidney, since the primary alteration may reside outside of the α2-adrenergic receptor. Further studies are needed to clarify the role of renal α1 adrenergic receptor sensitivity in both altered renal efferent sympathetic nerve activity and the pathogenesis of hypertension.

Acknowledgment

We thank Kathy Loppnow for secretarial assistance.

References


34. Sharma RV, Butters CA, Bhalla RC. Alterations in the plasma membrane properties of the myocardium of spontaneously hypertensive rats. Hypertension 1986;8:583–591


43. Takaku K, Itoh S, Kamasawa T, Takeda T. Protein kinase C activity in platelets from spontaneously hypertensive rats and normotensive Wistar Kyoto rats. Biochem Biophys Res Commun 1986;141:769–773


Renal alpha 1-adrenergic receptor response coupling in spontaneously hypertensive rats.
W B Jeffries, E Yang and W A Pettinger

_Hypertension_. 1988;12:80-88
doi: 10.1161/01.HYP.12.1.80
_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/12/1/80