Role of Renal Sympathetic Nerves in Regulating Renovascular Responses to Angiotensin II in Spontaneously Hypertensive Rats

John H. Dubinion, Zaichuan Mi, and Edwin K. Jackson

Center for Clinical Pharmacology (J.H.D., Z.M., E.K.J.), Departments of Pharmacology (J.H.D., E.K.J.) and Medicine (Z.M., E.K.J.), University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

Received January 11, 2006; accepted March 13, 2006

ABSTRACT

The purpose of this study was to test the hypothesis that renal sympathetic nerves modulate angiotensin II-induced renal vasoconstriction in kidneys from genetically hypertensive rats via Y1 receptors activating the Gi pathway. In isolated, perfused kidneys from spontaneously hypertensive rats, the naturally occurring renal sympathetic cotransmitter neuropeptide Y at 6 nM enhanced angiotensin II (0.3 nM)-induced changes in perfusion pressure by 47 ± 7 mm Hg, and this effect was inhibited by BIBP3226 [N(2-(diphenylacetyl)l-N-(4-hydroxyphenyl)-methyl)-D-arginine amide], a selective Y1 receptor antagonist (1 μM). We next examined whether periarterial nerve stimulation (5 Hz) enhances renal vascular responses to a physiological level of angiotensin II (100 pM). Kidneys were pretreated with prazosin (a selective α1-adrenoceptor antagonist) to block nerve stimulation-induced changes in perfusion pressure. In kidneys from spontaneously hypertensive rats, but not normotensive rats, periarterial nerve stimulation significantly augmented angiotensin II-induced changes in perfusion pressure (177 ± 26% of response in absence of stimulation). BIBP3226, but not rauwolscine (a selective α2-adrenoceptor antagonist), abolished periarterial nerve stimulation-induced enhancement of angiotensin II-mediated renal vasoconstriction. Pretreatment of hypertensive animals with pertussis toxin 3 days prior to kidney perfusion significantly (p < 0.000001) decreased mean blood pressure (203 ± 2 versus 145 ± 6 mm Hg in nonpretreated versus pertussis toxin-pretreated spontaneously hypertensive rats) and abolished periarterial nerve stimulation-induced enhancement of angiotensin II-mediated renal vasoconstriction. We conclude that, in spontaneously hypertensive rats but not normotensive rats, sympathetic nerve stimulation enhances renal vascular responses to physiological levels of angiotensin II via a mechanism mainly involving Y1 receptors coupled to Gi proteins.

In spontaneously hypertensive rats (SHR), but not in normotensive Wistar-Kyoto rats (WKY), activation of the Gi signal transduction pathway enhances renovascular responses to angiotensin (Ang) II via coincidence signaling involving the phospholipase C/protein kinase C/c-src/phosphatidylinositol 3-kinase pathway (Jackson et al., 2005). In this regard, concentrations of Gi pathway activators that do not alter basal renovascular resistance nonetheless markedly enhance renovascular responses to Ang II in SHR (Jackson et al., 2001, 2005; Gao et al., 2003).

Because Y1 receptors also activate the Gi signal transduction pathway (Berglund et al., 2003), we recently examined the effects of [Leu31,Pro34]-neuropeptide Y, a synthetic and highly selective Y1 receptor agonist, on Ang II-induced increases in renal vascular resistance in kidneys from SHR and WKY (Dubinion et al., 2006). As anticipated, this study showed that [Leu31,Pro34]-neuropeptide Y enhanced renovascular responses to Ang II in SHR, but not WKY, and that this effect was blocked by BIBP3226, a selective Y1 receptor antagonist, and by pertussis toxin, an inhibitor of the Gi signal transduction pathway.

The importance of the aforementioned observation is that renal sympathetic nerves corelease neuropeptide Y (NPY) (Dibona and Kopp, 1997), an endogenous, albeit nonselective, Y1 receptor agonist (Berglund et al., 2003). Therefore, it is conceivable that renal sympathetic nerves modulate Ang II-induced renal vasoconstriction in SHR kidneys via Y1 receptors activating the Gi pathway. The purpose of this study was to test this hypothesis.

ABBREVIATIONS: SHR, spontaneously hypertensive rat; WKY, Wistar Kyoto rat; Ang, angiotensin; BIBP3226, N(2-(diphenylacetyl)l-N-(4-hydroxyphenyl)methyl)-D-arginine amide; NPY, neuropeptide Y; UK14,304, 5-bromo-6[2-imidazoline-2-yl amino] quinoxaline; MABP, mean arterial blood pressure; RNS, renal nerve stimulation; P2/P1, ratio of the response to Ang II during period 2 to the response to Ang II during period 1; P3/P1, ratio of the response to Ang II during period 3 to the response to Ang II during period 1; SH-SP, hypertensive stroke-prone rats.
Materials and Methods

Animals. These studies used adult (14–16 weeks of age) male SHR and WKY rats. All rats were obtained from Taconic Farms (Germantown, NY), and the Institutional Animal Care and Use Committee approved all procedures. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (publication no. 85-23, revised 1996).

Drugs. Ang II, NPY, BIBP3226, and UK14,304 (a highly selective α2-adrenoceptor agonist) were obtained from Sigma (St. Louis, MO). All stated concentrations were calculated (nominal).

Isolation and Perfusion of Rat Kidneys. Rats were anesthetized with Inactin (100 mg/kg i.p.), and a catheter was inserted into the left carotid artery and connected to a digital blood pressure analyzer (model BPA; MicroMed, Louisville, KY) for measurement of mean arterial blood pressure (MABP) and heart rate in vivo. Next, the left kidney was removed from the animal and perfused in vitro. In brief, a polyethylene-10 tubing was inserted into the left ureter, and the left inferior suprarenal artery was ligated and cut. The aorta was cleared both above and below the left renal artery, and four sutures were placed loosely around the aorta, two above (sutures A and B) and two below (sutures C and D) the left renal artery. Sutures B and C were placed just above and below the left renal artery, respectively, and sutures A and D were placed approximately 1 cm above and below the left renal artery, respectively. Two additional sutures (E and F) were also placed loosely around the left renal vein with suture E positioned near the inferior vena cava and suture F near the renal hilus.

Once all sutures were in place, suture D on the lower aorta was tied off, and a vascular clamp was applied to the aorta just rostral to suture C. An incision was made in the aorta just rostral to suture D, and a polyethylene-50 tubing connected to the perfusion system was inserted into the aorta, advanced to the vascular clamp and secured in place with suture C. The vascular clamp was removed, the perfusion pump (see below) was activated to begin perfusion with Tyrode’s solution, and sutures A and B on the aorta above the renal artery were secured. Suture E on the renal vein near the inferior vena cava was tied off, and an incision was made in the renal vein. A catheter was placed in the renal vein and tied in place with suture F. The perfusion catheter in the aorta was advanced into the renal artery and secured in place with an additional suture on the renal artery. The aorta was severed between ties A and B and just rostral to tie D, and the renal vein was severed proximal to the renal venous catheter.

The left kidney was transferred rapidly to a Hugo Sachs Elektro-NIK-Harvard Apparatus GmbH (March-Hugstetten, Germany) kidney perfusion system. This system included the following components: Model UP 100 Universal Perfusion System, Model ISM 834 Channel Reglo Digital Roller Pump, a glass double-walled perfusate reservoir, a R 120144 glass oxygenator, mechanical integration of the oxygenator with the Universal Perfusion System UP 100, a Windkessel for absorption of pulsations, an inline holder for disc particle filters (80 μm), a temperature-controlled Plexiglass kidney chamber integrated with the UP 100, and a thermostatic circulator. The Plexiglass chamber contained a heat exchanger to maintain the temperature of the perfusate at 37°C at the point of entry into the tissue and also contained a device to extract bubbles from the perfusate just before the perfusate entered the kidney.

The Tyrode’s solution (137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl₂, 1.1 mM MgCl₂, 12 mM NaHCO₃, 0.42 mM NaH₂PO₄, and 5.6 mM d(+)-glucose, pH 7.4; osmolality, 280 mosM) was maintained at 37°C in the double-walled perfusate reservoir, bubbled with 95% oxygen/5% carbon dioxide, and pumped by the roller pump through a glass oxygenator (95% oxygen/5% carbon dioxide), through an inline particle filter, through an inline Windkessel, through a heat exchanger, through an inline bubble remover, and finally through the kidney. Kidneys were perfused (single-pass mode) at a constant flow (5 ml/min). Perfusion pressure was monitored continuously with a pressure transducer connected to digital blood pressure analyzer (model BPA).

Protocol 1. After the stabilization period, Ang II was infused to achieve a nominal concentration in the perfusate of 0.3 nM (period 1). We used this concentration to achieve robust responses to Ang II that could be easily quantified. After 10 min, the infusion of Ang II was stopped, and the perfusion pressure was allowed to return to basal levels over the next 10 min. Then, the following agents were infused to achieve the target concentration in the perfusate: 0.1 nM NPY, 1 nM NPY, 6 nM NPY, 10 nM UK14,304, 1 nM NPY + 10 nM UK14,304, or 6 nM NPY + 1 μM BIBP3226. In the case of those kidneys treated with BIBP3226 (a highly selective Y1 receptor antagonist; Berglund et al., 2003), the antagonist was infused beginning from the outset of perfusion (i.e., during the stabilization period) so that the kinetics of displacement of the agonist by the antagonist would not confound data interpretation. Because a given kidney received only one treatment, this protocol represented six distinct groups with six to seven kidneys per group (total of 37 kidney perfusion experiments). Twenty minutes into the treatments, the kidney was restimulated with Ang II for 10 min (period 2). By using only a low concentration of Ang II and by limiting the experiment to two challenges with Ang II, tachyphylaxis to Ang II was avoided and responses to Ang II were stable. The response to Ang II was taken as the change in perfusion pressure during the Ang II infusion and was calculated as the perfusion pressure recorded at the end of the infusion of Ang II minus the basal perfusion pressure recorded just before the Ang II infusion.

Protocol 2. Experiments were conducted in 66 isolated, perfused rat kidneys. Immediately after initiating perfusion of the kidney, a platinum bipolar electrode was placed around the renal artery for renal nerve stimulation (RNS). The electrode was connected to a Grass stimulator (model SD9E; Grass Instruments, Quincy, MA). After a 60-min rest period, prazosin (30 nM) was added to the perfusate to block α1-adrenoceptors so that RNS would not cause direct vasoconstriction and increase basal vascular tone. This was necessary because changes in basal vascular tone might nonspecifically elevate responses to Ang II. Ten minutes after adding prazosin to the perfusate, RNS was simulated by going through the motions of activating the stimulator while not actually activating the stimulator (sham RNS). Two minutes into the sham RNS, Ang II was infused into the renal artery for 7 min to provide a final concentration of 100 pM, which is a physiological level of Ang II in SHR as recently determined by capillary electrophoresis (Sim and Qui, 2003). This concentration was selected because, although it was a lower concentration than used in protocol 1, it allowed us to conduct experiments with physiological levels of Ang II. This first response to Ang II was designated period 1. At the end of the Ang II infusion, the kidney was allowed a rest period of 10 min and then the kidney was subjected to either sham RNS or RNS (biphasic, 5 Hz, 1-ms pulse duration, 35 V) for 9 min. Two minutes into the sham RNS or RNS, Ang II was infused once again for 7 min to provide a final concentration of 100 pM. This second response to Ang II was designated period 2. Next, the periarterial electrodes were repositioned, and after another 10-min rest period, a third response to Ang II was obtained in the absence (sham RNS) or presence (RNS) of renal sympathetic activation (period 3). Kidneys were randomly assigned to receive either sham RNS during periods 2 and 3 or active RNS during periods 2 and 3.

In some experiments, either rauwolscine or BIBP3226 was added to the perfusate at the beginning of kidney perfusion. In addition, some kidneys were removed from rats that had been pretreated 3 days earlier with an i.v. injection of pertussis toxin (30 μg/kg) to block Gi proteins as described previously by us (Jackson, 1994). Kidneys were randomly assigned to no inhibitor or rauwolscine or BIBP3226.

The vasoconstrictor response to Ang II was assessed by the change in perfusion pressure at the point of entry into the tissue and also contained a device to extract bubbles from the perfusate just before the perfusate entered the kidney.
in renal perfusion pressure in this constant flow system. The ability of RNS to enhance vasoconstrictor responses to Ang II during periods 2 and 3 was determined by calculating the ratios of the response to Ang II during period 2 to the response to Ang II during period 1 (P2/P1) and the ratio of the response to Ang II during period 3 to the response to Ang II during period 1 (P3/P1). Time-dependent changes in responses to Ang II were assessed by calculating the same ratios in the sham RNS groups.

Statistics. In protocol 1, vasoconstrictor responses to Ang II were compared between periods 1 and 2 by a paired two-tailed Student’s t test. For protocol 2, statistical comparisons were between P2/P1 for sham RNS groups versus P2/P1 for RNS groups and between P3/P1 for sham RNS groups versus P3/P1 for RNS groups. Because the data did not violate test assumptions of normality, comparisons were performed with an unpaired two-tailed Student’s t test. Statistical analysis was conducted using NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, UT). The criterion of significance was p < 0.05. All values in text and tables are means ± S.E.M.

Results

Protocol 1. The average baseline (i.e., in the absence of Ang II) renal perfusion pressure in these 37 experiments was 45 ± 1 mm Hg and was not affected by any of the various treatments or combinations of treatments. For periods 1 and 2, respectively, baseline perfusion pressures were: for 0.1 nM NPY, 58 ± 5 and 49 ± 4 mm Hg; for 1 nM NPY, 43 ± 2 and 39 ± 2 mm Hg; for 6 nM NPY, 43 ± 1 and 42 ± 1 mm Hg; for 6 nM NPY + 1 µM BIBP3226, 44 ± 3 and 40 ± 2 mm Hg; for UK14,304, 41 ± 2 and 39 ± 2 mm Hg; and for 1 nM NPY + 10 nM UK14,304, 43 ± 4 and 41 ± 3 mm Hg. Although the perfusion flow rate was physiological (5 ml/min), baseline perfusion pressures were below the normal renal perfusion pressure in vivo because of the low viscosity of Tyrode’s solution compared with whole blood. However, despite the low basal perfusion pressure, the kidneys were extremely responsive to Ang II.

As shown in Fig. 1A, 0.1 nM NPY did not potentiate renovascular responses to Ang II. Although 1 nM NPY did significantly enhance renovascular responses to Ang II, this effect was small (only 4 ± 2 mm Hg; Fig. 1B). In contrast, 6 nM NPY more than doubled the renovascular response to Ang II (Fig. 1C). Higher concentrations of NPY were not examined because, although concentrations equal to or less than 6 nM did not change baseline perfusion pressure, higher concentrations did. As shown in Fig. 1D, in kidneys treated with BIBP3226, 6 nM NPY did not significantly augment renovascular responses to Ang II. Although our previously published studies demonstrated that 10 nM UK14,304 augmented renovascular responses to 10 nM Ang II (Gao et al., 2003), as shown in Fig. 1E, 10 nM UK14,304 did not significantly enhance renovascular responses to a very low concentration of Ang II (0.3 nM). However, when an ineffective concentration of UK14,304 (10 nM) was combined with a concentration of NPY (1 nM) that only slightly potentiated responses to Ang II (by 4 ± 2 mm Hg), a marked potentiation of the renovascular response to Ang II was observed (24 ± 7 mm Hg, p = 0.0240 compared with the potentiation observed for 1 nM NPY alone).

Protocol 2. As shown in Table 1, MABPs were significantly higher in SHR compared with WKY, and pretreatment with pertussis toxin significantly reduced MABP in SHR. Basal renal perfusion pressures, either before or after prazosin, were similar among all 10 groups. Sham RNS did not affect renal perfusion pressure, and actual RNS did not affect renal perfusion pressure in the presence of prazosin because prazosin blocked α1-adrenoceptor-induced vasoconstriction.

Figure 2 demonstrates the lack of effect of RNS on renovascular responses to Ang II in WKY kidneys. During P1, a physiological concentration of Ang II (100 pM) increased renal perfusion pressure by 1.6 ± 0.2 mm Hg in WKY kidneys. This response to Ang II did not change during P2 or P3 in either the sham RNS group or the actual RNS group.

Figure 3 illustrates the ability of RNS to enhance renovascular response to Ang II in SHR kidneys. During P1, Ang II (100 pM) increased renal perfusion pressure by 8.6 ± 0.7 mm Hg in SHR kidneys. This response to Ang II did not change during P2 or P3 in either the sham RNS group or the actual RNS group.

Figure 4 shows that the ability of RNS to enhance renovascular response to Ang II in SHR kidneys was not blocked by rauwolscine, a highly potent and selective α2-adrenoceptor antagonist. In kidneys pretreated with rauwolscine, during P1, Ang II (100 pM) increased renal perfusion pressure by 6.3 ± 0.7 mm Hg in SHR kidneys. This response to Ang II did not change during P2 or P3 in the sham RNS group; however,
in the actual RNS group, the response to Ang II was increased to 132 ± 9% and 147 ± 7% (normalized to the P1 response) (p < 0.05). Moreover, the enhancement of renovascular responses to Ang II by RNS in SHR kidneys treated with rauwolscine was not statistically different (unpaired Student’s t test) from the enhancement observed in SHR kidneys not treated with rauwolscine (comparisons between upper curves in Fig. 3 versus Fig. 4).

Figure 5 demonstrates that the ability of RNS to enhance renovascular response to Ang II in SHR kidneys is completely abrogated by BIBP3226, a highly selective and potent Y₁ receptor antagonist. In kidneys pretreated with BIBP3226, during P1, Ang II (100 pM) increased renal perfusion pressure by 7.6 ± 0.7 mm Hg in SHR kidneys. This response to Ang II did not change during P2 or P3 in either the sham RNS group or the actual RNS group.

Figure 6 demonstrates that the ability of RNS to enhance renovascular response to Ang II in SHR kidneys is completely abrogated by pertussis toxin, a toxin that ADP ribosylates Gi proteins and thereby inhibits signaling via Gi proteins. In kidneys obtained from SHR pretreated with pertussis toxin, during P1, Ang II (100 pM) increased renal perfusion pressure by 7.6 ± 0.7 mm Hg in SHR kidneys. This response to Ang II did not change during P2 or P3 in either the sham RNS group or the actual RNS group.

Discussion

This study tested the hypothesis that renal sympathetic nerves modulate Ang II-induced renal vasoconstriction in SHR kidneys via Y₁ receptors activating the G₁ pathway. To test this hypothesis, it was important to examine the effects
Values are means \( \pm \) S.E.M. Some kidneys were exposed only to sham RNS (SHAM). Ang II during P2 and P3 were normalized to the responses during the control P1. Some kidneys were exposed only to sham RNS (SHAM). Values are means \( \pm \) S.E.M.

**Fig. 4.** Periarterial RNS enhances vasoconstrictor responses (change in renal perfusion pressure in a constant flow system) to Ang II (100 pM) in isolated, perfused kidneys obtained from SHRs that were pretreated with the \( \alpha_2 \)-adrenoceptor antagonist rauwolscine (10 nM). The responses to Ang II during P2 and P3 were normalized to the responses during the control P1. Some kidneys were exposed only to sham RNS (SHAM).

**Fig. 5.** Periarterial RNS does not enhance vasoconstrictor responses (change in renal perfusion pressure in a constant flow system) to Ang II (100 pM) in isolated, perfused kidneys obtained from SHRs that were pretreated with the \( \gamma_1 \)-receptor antagonist BIBP3226 (1 \( \mu \)M). The responses to Ang II during P2 and P3 were normalized to the responses during the control P1. Some kidneys were exposed only to sham RNS (SHAM). Values are means \( \pm \) S.E.M.

**Fig. 6.** Periarterial RNS does not enhance vasoconstrictor responses (change in renal perfusion pressure in a constant flow system) to Ang II (100 pM) in isolated, perfused kidneys obtained from SHRs that were pretreated 3 days prior to the perfusion experiment with an i.v. injection of pertussis toxin (30 \( \mu \)g/kg). The responses to Ang II during P2 and P3 were normalized to the responses during the control P1. Some kidneys were exposed only to sham RNS (SHAM). Values are means \( \pm \) S.E.M.

The mechanism of this interaction between \( \gamma_1 \)-adrenoceptors and NPY was not examined in the current study; however, because both NPY-sensitive receptors (Berglund et al., 2003) and \( \alpha_2 \)-adrenoceptors (Jackson et al., 2005) stimulate the G\(_i\) pathway, it is likely that the synergy is meditated by the convergence of these two signaling pathways on G\(_i\) to generate more effective activation of this G protein.

Our hypothesis predicts that the ability of NPY to augment renovascular responses to Ang II is mediated by \( \gamma_1 \) receptors. Consistent with this prediction, BIBP3226 abolishes NPY-induced potentiation of Ang II-stimulated renal vasoconstriction. This finding firmly establishes the \( \gamma_1 \) receptor as mediating this important action of NPY.

If our hypothesis is correct, sympathetic nerve stimulation should augment renovascular responses to physiological levels of Ang II in SHR, but not WKY, kidneys. In this regard, our results confirm that stimulation of the renal periarterial nerves results in a near doubling of the renovascular response to a physiological level (100 pM) of Ang II in kidneys from SHR. In contrast, in kidneys from WKY rats, periarterial nerve stimulation does not significantly alter vasoconstrictor responses to Ang II. Thus, we conclude that, in kidneys from genetically hypertensive rat but not kidneys from normotensive rats, sympathetic nerve stimulation enhances renovascular responses to physiological levels of Ang II.

Our previous studies show that activation of the GI pathway by agonists of \( \alpha_2 \)-adrenoceptors also potentiated renovascular response to Ang II in SHR kidneys, but not WKY, kidneys (Jackson et al., 2001, 2005; Gao et al., 2003). Because renal sympathetic nerves release both norepinephrine and NPY (Dibona and Kopp, 1997), which activate \( \alpha_2 \)-adrenoceptors and \( \gamma_1 \) receptors, respectively, it is possible that the ability of renal sympathetic nerve stimulation to augment renovascular responses to Ang II is due, at least in part, to activation of \( \alpha_2 \)-adrenoceptors. However, in the present study, rauwolscine (an \( \alpha_2 \)-adrenoceptor antagonist) failed to affect significantly the ability of periarterial nerve stimulation to augment Ang II-induced renal vasoconstriction. This lack of effect of rauwolscine was not likely due to inadequate
concentrations of rauwolscine because 10 nM rauwolscine (the concentration used in the present study) was shown to affect α₂-adrenoceptors in the isolated perfused rat kidney (Fujimura et al., 1999), and the Kᵦ of rauwolscine for rat kidney membranes is approximately 1 nM (Lanier et al., 1987). In contrast to rauwolscine, BIBP3226 (a Y₁ receptor antagonist) completely abrogated nerve stimulation-enhanced formation of Ang II renovascular responses, a finding entirely consistent with a NPY-mediated action on Y₁ receptors. A possible explanation for this finding is that norepinephrine mainly undergoes prejunctional and postjunctional uptake and therefore may have limited access to α₂-adrenoceptors, which are known to be primarily extrajunctional (Pettinger et al., 1987). On the other hand, NPY does not undergo uptake and would be available to diffuse to both junctional and extrajunctional Y₁ receptors. If this hypothesis is correct, this leaves open the possibility that circulating catecholamines, for example from the adrenal gland, may also enhance renovascular responses to Ang II in SHR under the appropriate conditions, either per se or in synergy with NPY. Additional studies are required to determine whether circulating catecholamines may influence the ability of neuronally released NPY to enhance renovascular responses to Ang II in SHR kidneys.

The hypothesis that renal sympathetic nerves modulate Ang II-induced renal vasoconstriction in SHR kidneys via Y₁ receptors activating the Gᵢ pathway has important implications regarding the pathophysiology of hypertension in SHR. In this regard, numerous studies show that the renin-angiotensin system is critical for the development and maintenance of high blood pressure in SHR (Ferrone and Antonaccio, 1979; Bunckenburg et al., 1991). Importantly, transplantation studies demonstrate that the SHR kidney is also essential to the pathophysiology of hypertension in the SHR (Rettig and Unger, 1991; Grisk et al., 2002), and other investigations demonstrate that the renal sympathetic nervous system contributes to the pathophysiology of hypertension in SHR (Winternitz et al., 1980; Norman and Dzielak, 1982). However, a coherent hypothesis that explains the co-involvement of the renin-angiotensin system, the sympathetic nervous system, and the kidneys in SHR hypertension is lacking.

Why is there a co-involvement of the renin-angiotensin system, the renal sympathetic system, and the kidneys in SHR hypertension? Studies do not support an enhanced expression of renal AT₁ receptors (Gao et al., 2003) or increased circulating levels of Ang II (Shiono and Sokabe, 1976) or increased local kidney levels of Ang II (Campbell et al., 1995). Moreover, SHRs do not have reduced renal Ang II degradation rates (Jackson and Herzer, 2003). On the other hand, SHRs do exhibit increased renovascular responses to Ang II (Li and Jackson, 1989; Kost and Jackson, 1993), and this could explain the co-involvement of the RAS and the kidney in SHR hypertension. The results of the present study demonstrate that renal sympathetic nerves enhance renovascular responses to Ang II in SHR, but not WKY, kidneys, and this finding provides a critical link that connects the three physiological systems (i.e., renal system, renin-angiotensin system, and renal sympathetic nervous system). This unifying hypothesis proposes that hypertension in SHR is due in part to a genetic abnormality in the renal microcirculation that allows the renal sympathetic nerves to potentiate Ang II-induced renal vasoconstriction, thus causing long-term changes in renal function.

The results of the present study show that blockade of Gᵢ with pertussis toxin abolishes the ability of renal sympathetic nerve stimulation to enhance renovascular responses to Ang II in SHR kidneys. This finding indicates that renal sympathetic nerve stimulation augments renovascular responses to Ang II in SHR kidneys via a Gᵢ signal transduction pathway. Importantly, our previous research indicates that the Gᵢ signal transduction mechanism mediates in part the enhanced renovascular response to Ang II in SHR. For example, pertussis toxin, an inhibitor of Gᵢ, abolishes the increased renovascular response to Ang II in SHR (Jackson, 1994). The fact that pertussis toxin blocks both the enhanced renovascular responses to Ang II in SHR kidneys in vivo and the ability of renal sympathetic nerves to augment Ang II-induced renal vasoconstriction in vitro is critical support for the hypothesis that enhanced renovascular responses to Ang II in SHR are mediated in part via activation of renal sympathetic nerves. Published studies by us (Kost et al., 1999) and others (Li and Anand-Srivastava, 2002) demonstrate that pertussis toxin is antihypertensive in SHR, and the results of the present study are consistent with that conclusion. The antihypertensive action of pertussis toxin in SHR is also consistent with our aforementioned unifying hypothesis regarding the pathophysiology of hypertension in SHR.

The fact that renal sympathetic nerve stimulation enhances renovascular responses to Ang II in SHR, but not WKY, could be due to augmented levels of NPY, increased Y₁ receptor expression, or increased coincidence signaling via the Gᵢ pathway. Numerous investigators have examined NPY levels in kidneys from SHR and WKY and found no differences (Zukowska-Grojec et al., 1993; Pavia and Morris, 1994; Corder, 2000). These data argue strongly against the notion that SHR kidneys store or release more NPY. We previously measured Y₁ receptor levels in renal microvessels and kidneys from SHR and WKY kidneys and found no difference in expression (Dubinion et al., 2006), thus ruling out a role for differences in Y₁ receptor expression. Most likely then, the greater role for NPY/Y₁ receptors in augmenting renovascular responses to Ang II in SHR is due to greater coincidence signaling downstream of the Y₁ receptor. Indeed, previously we compared the effects of a specific exogenous Y₁ receptor agonist in SHR versus WKY kidneys (Dubinion et al., 2006) and observed that the effects of the agonist were greater in SHR compared with WKY at the same concentration of agonist. The fact that the defect in the SHR renal microcirculation can be demonstrated with different kinds of activators of the Gᵢ pathway (Jackson et al., 2001, 2005; Gao et al., 2003; Dubinion et al., 2006) strongly supports a defect downstream of both storage/release of NPY and the Y₁ receptor.

Recently, Vonend et al. (2005) examined the role of endogenously released NPY in hypertensive stroke-prone rats (SH-SP) compared with WKY with regard to mediating the direct effects of RNS on renal vascular resistance in isolated kidneys. These authors found that neuronally released NPY-potentiated RNS-induced nonadrenergic neurotransmission in WKY and young SH-SP, but not in adult SH-SP. These findings are not at odds with our results because our experiments focused on the interaction between endogenous NPY
and Ang II, rather than endogenous NPY and nonadrenergic neurotransmission.

There are several caveats regarding the interpretation of our experiments. First, we used a frequency of periartrial nerve stimulation of 5 Hz, which is higher than the generally accepted basal, physiological frequency of 0.5 to 2.5 Hz for renal sympathetic nerves (Dibona and Kopp, 1997). This frequency was selected because NPY release occurs most robustly at high intensities of sympathetic activation (Lundberg et al., 1991). However, the notion of a “physiological frequency” of renal sympathetic nerve activity has limited utility because renal sympathetic nerve discharge is not coherent, involves multiple postganglionic sympathetic fibers depolarizing at different frequencies and different phases, and engages some fibers to discharge in short-duration bursts of activity that have high frequency within a short time domain. Indeed, spectral analysis of renal sympathetic nerve activity shows a range of frequencies in the power spectrum from 0.5 to 10 Hz (Malpas, 1998).

Another caveat is that the experiments were not conducted in the presence of drugs to block the release of renin by periartrial nerve stimulation. Therefore, it is possible that the enhancement of vasoconstrictor responses to exogenous Ang II by periartrial nerve stimulation was mediated by endogenous Ang II formed by renin. However, this seems unlikely because such a mechanism would not be blocked by BIBP3226 and would be augmented, rather than inhibited, by pertussis toxin because pertussis toxin increases renin release (Pedraza-Chaverri et al., 1986).

A third caveat is that the isolated perfused rat kidney is not physiological because of low viscosity of the perfusate, lack of osmotic colloid in the perfusate, and relative hypoxia. However, we have examined the histology of kidneys perfused in our laboratory using hematoxylin-eosin, periodic acid-Schiff, and methenamine silver-trichrome stains, and we do not observe deterioration in structural integrity of vessels, glomeruli, or tubules for up to 5 h of perfusion, and the vasculature responds to Ang II, norepinephrine, methoxamine, vasopressin, and sympathetic nerve stimulation.

A fourth caveat is that the general toxic effects of pertussis toxin account for the results we obtained with this pharmacological probe. This is unlikely, however, because pertussis toxin did not modify the basal response to Ang II in SHR kidneys, and we previously showed that pertussis toxin has minimal effects on blood pressure in WKY rats (Kost et al., 1999).

The renal sympathetic nervous system and renin-angiotensin system interact at multiple levels to regulate renal vascular tone and, hence, long-term levels of arterial blood pressure. Augmentation of any of these interactions could contribute to the pathophysiology of high blood pressure. The present experiments demonstrate that, in SHR but not WKY kidneys, renal sympathetic nerve stimulation augments the ability of Ang II to constrict the renal circulation. The mechanism of this augmentation involves Y1 receptors signaling via the Gi transduction pathway. Previous studies by many investigators implicate the renal system, the renin-angiotensin system, and the sympathetic nervous system in the pathophysiology of genetic hypertension. The implications of the experiments reported here provide a possible explanation for the co-occurrence of these three systems in genetic hypertension.

References

Address correspondence to: Dr. Edwin K. Jackson, Center for Clinical Pharmacology, 100 Technology Drive, Suite 450, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219. E-mail: edij@pitt.edu.