Pancreatic Polypeptide-Fold Peptide Receptors and Angiotensin II–Induced Renal Vasoconstriction

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Abstract—The \( G_i \) pathway augments renal vasoconstriction induced by angiotensin II in spontaneously hypertensive but not normotensive Wistar-Kyoto rats. Because the \( G_i \)-coupled pancreatic polypeptide (PP)-fold peptide receptors \( Y_1 \) and \( Y_2 \) are expressed in kidneys and are activated by endogenous PP-fold peptides, we tested the hypothesis that these receptors regulate angiotensin II-induced renal vasoconstriction in kidneys from hypertensive but not normotensive rats. A selective \( Y_1 \)-receptor agonist [(Leu31,Pro34)-neuropeptide Y; 6 to 10 nmol/L] greatly potentiated angiotensin II–induced changes in perfusion pressure in isolated, perfused kidneys from hypertensive but not normotensive rats. A selective \( Y_2 \)-receptor agonist (peptide YY3-36; 6 nM) only slightly potentiated angiotensin II–induced renal vasoconstriction and only in kidneys from hypertensive rats. Neither the \( Y_1 \)-receptor nor the \( Y_2 \)-receptor agonist increased basal perfusion pressure. BIBP3226 (1 \( \mu \text{mol/L} \), highly selective \( Y_2 \)-receptor antagonist) and BIIE0246 (1 \( \mu \text{mol/L} \), highly selective \( Y_2 \)-receptor antagonist) completely abolished potentiation by (Leu31,Pro34)-neuropeptide Y and peptide YY3-36, respectively. \( Y_1 \)-receptor and \( Y_2 \)-receptor mRNA and protein levels were expressed in renal microvessels and whole kidneys, but the abundance was similar in kidneys from hypertensive and normotensive rats. Both \( Y_1 \)-receptor–induced and \( Y_2 \)-receptor–induced potentiation of angiotensin II–mediated renal vasoconstriction was completely abolished by pretreatment with pertussis toxin (30 \( \mu \text{g/kg IV} \), blocks \( G_i \) proteins). These data indicate that, in kidneys from genetically hypertensive but not normotensive rats, \( Y_1 \)-receptor activation markedly enhances angiotensin II–mediated renal vasoconstriction by a mechanism involving \( G_i \). Although \( Y_2 \) receptors can also potentiate angiotensin II–mediated renal vasoconstriction via \( G_i \), the effect is modest compared with \( Y_1 \) receptors. These findings may have important implications for the etiology of genetic hypertension. (Hypertension. 2006;47[part 2]:545-551.)

Key Words: receptors ■ neuropeptides ■ peptides ■ hypertension

The renin–angiotensin system (RAS) is essential for the development and maintenance of genetic hypertension in spontaneously hypertensive rats (SHRs).\(^1,2\) Moreover, transplantation studies reveal that, in addition to the RAS, the SHR kidney is pivotal to the pathophysiology of hypertension in the SHR.\(^3,4\) Finally, the renal sympathetic nervous system also appears to importantly contribute to the pathophysiology of hypertension in SHRs. In support of this latter concept, chronic denervation of the SHR kidney both delays the development of hypertension and attenuates the maximum increase in blood pressure in SHR.\(^5-8\) Thus, there appears to be a coinvolvement of the RAS, the sympathetic nervous system, and the kidney in SHR hypertension.

Many studies have been performed in search of a possible explanation for the coinvolvement of the RAS and the kidney in SHR hypertension. In this regard, studies do not support an increased expression of renal angiotensin II (Ang II) receptors\(^9\) or increased levels of circulating\(^10\) or renal\(^11\) Ang II; also, SHRs do not have altered renal Ang II degradation rates.\(^12\) However, SHRs do exhibit increased renovascular responses to Ang II,\(^13,14\) and this appears to be the explanation for the coinvolvement of the RAS and the kidney in SHR hypertension.

Our previous research indicates that \( G_i \) mediates, in part, the enhanced renovascular response to Ang II in SHR. For example, pertussis toxin, an inhibitor of \( G_i \), abolishes the increased renovascular response to Ang II in SHR.\(^15,16\) Importantly, activation of renal sympathetic nerves leads to the release of neuropeptide Y (NPY).\(^17\) NPY is an example of a pancreatic polypeptide (PP)-fold peptide,\(^18\) and NPY binds with high affinity to \( Y_1 \), \( Y_2 \), and \( Y_5 \) receptors but not \( Y_4 \) receptors,\(^19\) whereas \( Y_1 \) receptors most likely do not exist.\(^19\) Both \( Y_1 \) and \( Y_5 \) receptors are expressed in the kidney,\(^17,18\) whereas \( Y_5 \) receptors are expressed predominantly in the central nervous system, not the kidney.\(^20\) Because \( Y_1 \) and \( Y_5 \) receptors coupled to \( G_i \) exist in the kidney and are stimulated by NPY, it is conceivable that the coinvolvement of the RAS, sympathetic nervous system, and kidney in SHR hypertension is mediated in part by activation of \( Y_1 \) and/or \( Y_5 \) receptors leading to a potentiation of Ang II–induced renal vasoconstriction in the SHR kidney.
The purpose of the present study was to test the hypothesis that Y1 and/or Y2 receptor activation potentiates renal vascular responses to Ang II selectively in SHR kidneys by a mechanism involving inhibitory G proteins. In this regard, we examined the effects of (Leu31,Pro34)-NPY (LPNPY), a highly selective Y1 agonist, and peptide Y1<sub>3-36</sub> (PY1<sub>3-36</sub>), a highly selective Y1 agonist, on renovascular responses to Ang II in SHR and Wistar-Kyoto (WKY) kidneys in vitro in the absence and presence of a highly selective Y1 antagonist (BIBP3226<sup>18</sup>), a highly selective Y2 antagonist (BIIE0246<sup>18</sup>), or pertussis toxin to inhibit G proteins.

Methods

Animals

Studies used adult (14 to 16 weeks of age) male SHRs or WKY obtained from Taconic Farms (Germantown, NY). Some SHRs were pretreated 3 days before the experiment with an intravenous (tail vein) injection of pertussis toxin (30 mg/kg given as a bolus; Sigma). We have shown previously that this approach blocks G-protein function in vivo. 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 National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Experiments in Isolated, Perfused Kidneys

SHRs and WKY were anesthetized with Inactin (90 mg/kg, IP), and the left kidney was isolated and perfused with Tyrode’s solution using a Hugo Sachs Electronik-Harvard Apparatus GmbH (March-Hugstetten) kidney perfusion system as described previously. Briefly, all branches of the left renal artery and vein were ligated. A polyethylene-50 cannula was placed into the left renal artery, and a polyethylene-90 cannula was placed into the left renal vein. The left kidney was removed, attached to the perfusion system, and allowed to stabilize for 1 hour before the experimental protocol. Kidneys were perfused (single pass mode) at a constant flow (5 mL/min), and perfusion pressure was monitored with a pressure transducer.

In 1 study, Ang II (Sigma) was infused at increasing doses to provide concentrations in the perfusate of 0.3, 1, or 3 nmol/L. Each dose of Ang II was infused for 2 minutes, and the perfusion pressure was allowed to return to basal levels over the next 5 minutes before initiating the next higher dose of Ang II. After the highest dose of Ang II and following a rest period of 15 minutes, LPNPY (Sigma) was infused into the kidney to provide a concentration in the perfusate of 10 nmol/L. Ten minutes into the infusion of LPNPY, the kidney was restimulated with Ang II. 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 infusion of Ang II. Time control experiments demonstrated that concentration-response curves to Ang II were stable over 2 minutes, which avoided tachyphylaxis because of multiple challenges with Ang II, tachyphylaxis to Ang II was avoided, and responses to Ang II were stable. Changes in perfusion pressure in response to Ang II were calculated as described above. Some of these experiments were conducted in kidneys in which BIBP3226 (Tocris) or BIIE0246 (Tocris) was infused into the perfusate to provide a concentration of 1 μmol/L. The infusions of BIBP3226 or BIIE0246 were initiated at the beginning of the 1-hour rest period and continued until the end of the protocol.

RT-PCR for Y<sub>1</sub> and Y<sub>2</sub> Receptor mRNA and Western Blotting for Y<sub>1</sub> and Y<sub>2</sub> Receptor Protein

Y<sub>1</sub> or Y<sub>2</sub> receptor mRNA was obtained from SHR and WKY preglomerular microvessels (PGMVs) and whole kidneys and measured as described previously for other receptors. Briefly, PGMVs were obtained by iron oxide injection into the renal artery followed by magnetic retrieval of PGMVs. Total RNA was isolated from PGMVs or whole kidney with TRIzol reagent. RNA was reverse transcribed and amplified using a Titanium One-Step RT-PCR kit. For the Y<sub>1</sub> receptor, the forward primer was 5’-CTGATCGTG-AACCTCCTCTTCT-3’, and the reverse primer was 5’-GTCGTGTAAGACAGGCTGTGAG-3’. For the Y<sub>2</sub> receptor, the forward primer was 5’-GGTCTGCTCAGGCAATGCTCC-3’, and the reverse primer was 5’-GTCGTTTTCTGGCTTCGTCG-3’. Each PCR cycle consisted of denaturing at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 60 seconds. RT-PCR products were separated on a 1.2% agarose gel, and gels were stained with ethidium bromide.

Statistical Analysis

Data were analyzed by paired or unpaired 2-tailed Student t test or 1-factor or 2-factor ANOVA, as appropriate. The Fisher least significant difference (LSD) test was used for post-hoc analyses if a significant ANOVA was obtained. The criterion of significance was P<0.05. All of the data are presented as mean±SEM.

Results

Basal Renal Perfusion Pressures

In this study, baseline renal perfusion pressures were similar (60±3 and 52±2 mm Hg) in WKY and SHR kidneys, respectively, and were not affected by any of the various treatments or combinations of treatments. 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 very responsive to Ang II.

Experiments in Isolated, Perfused Kidneys

With LPNPY

As shown in Figure 1, 0.3 (Figure 1A), 1 (Figure 1B), and 3 (Figure 1C) nmol/L of Ang II caused a concentration-
dependent increase in renal perfusion pressure in both WKY (left graphs) and SHRs (right graphs). Ang II–induced increases in renal perfusion pressure were significantly greater in SHR compared with WKY kidneys (right versus left graphs in Figure 1). In SHR kidneys and at all concentrations of Ang II, LPNPY (10 nmol/L) significantly potentiated Ang II–induced increases in renal perfusion pressure (Figure 1, right graphs). In contrast, in WKY kidneys, LPNPY did not augment Ang II–induced increases in renal perfusion pressure regardless of the concentration of Ang II (Figure 1, left graphs). Two-factor ANOVA demonstrated a significant interaction between rat strain and LPNPY on Ang II–induced changes in perfusion pressure ($P<0.003$, $P<0.001$, and $P<0.004$ for 0.3, 1, and 3 nmol/L Ang II, respectively).

In a separate set of experiments using naïve SHR kidneys, LPNPY (6 nmol/L) again markedly and significantly potentiated the ability of Ang II (at 0.3 nmol/L infused to a steady-state response) to increase renal perfusion pressure (Figure 2A). The ability of LPNPY to potentiate Ang II–induced renal vasoconstriction was greatly suppressed by both BIBP3226 (Figure 2B) and pertussis toxin (Figure 2C); however, there remained a statistically significant, albeit small, residual potentiation. LPNPY enhanced Ang II–induced changes in perfusion pressure by 54±8, 8±3, and 13±6 mm Hg in untreated, BIBP3226-treated and pertussis toxin–treated kidneys, respectively, and the ability of LPNPY to enhance Ang II–induced changes in perfusion pressure was statistically significantly suppressed by both BIBP3226 and pertussis toxin.

**RT-PCR for Y₁ Receptor mRNA and Western Blotting for Y₁ Receptor Protein**

As shown in Figure 3, mRNA and protein expression for Y₁ receptors was detected in whole kidney and PGMVs from both WKY and SHRs. However, the expression of Y₁...
receptor mRNA and protein, when normalized to β-actin and quantified by densitometry, did not differ between WKY and SHR tissues (Figure 4).

Experiments in Isolated, Perfused Kidneys

With PYY3-36

As shown in Figure 5A, PYY3-36 caused a very small but statistically significant and reproducible potentiation of renal vasoconstriction induced by Ang II (0.3 nmol/L infused to steady-state response; 32 ± 5 and 41 ± 5 mm Hg, change in perfusion pressure in response to Ang II before and during PYY3-36, respectively). However, in WKY, PYY3-36 did not significantly alter Ang II–induced renal vasoconstriction (17 ± 5 and 14 ± 3 mm Hg, change in perfusion pressure in response to Ang II before and during PYY3-36, respectively).

As shown in Figure 5, BIIE0246 (Figure 5B) and pertussis toxin (Figure 5C) completely blocked PYY3-36-induced potentiation of Ang II–induced changes in perfusion pressure. PYY3-36 enhanced Ang II–induced changes in perfusion pressure by 9 ± 2, −2 ± 2, and −1 ± 3 mm Hg in untreated, BIIE0246-treated, and pertussis toxin–treated kidneys, respectively, and the ability of PYY3-36 to enhance Ang II–induced changes in perfusion pressure was statistically significantly suppressed by both BIIE0246 and pertussis toxin.

RT-PCR for Y2 Receptor mRNA and Western Blotting for Y2 Receptor Protein

As shown in Figure 6, mRNA and protein expression for Y2 receptors was detected in whole kidney and PGMVs from both WKY and SHRs. However, the expression of Y2 receptor mRNA and protein, when normalized to β-actin and quantified by densitometry, did not differ between WKY and SHR tissues (data not shown).

Discussion

The PP-fold family of peptides consists of 4 members, NPY, PYY1-36, PYY3-36, and PP.18,19 Four active PP-fold peptide receptors have been cloned in primate species, namely Y1, Y2, Y4, and Y5.18,19 Although, as confirmed in the present study, Y1 and Y2 receptors are expressed in the kidney, Y4 and Y5 receptors are expressed predominantly in the intestines and brain,18 not the kidney. Therefore, in the present study, we focused on the role of Y1 and Y2 receptors as regulators of Ang II–induced renal vasoconstriction.

Figure 3. RT-PCR products for Y1 receptor mRNA and β-actin mRNA in WKY and SHR kidneys (top left) and in preglomerular microvessels (PGMV; bottom left). Western blot analysis of Y1 receptor and β-actin in WKY and SHR kidneys (top right) and preglomerular microvessels (PGMV; bottom right).

Figure 4. Top: densitometry results (normalized to β-actin signal) from Western blot analysis of Y1 receptor protein in WKY vs SHR kidneys (left) and preglomerular microvessels (PGMV; right). Bottom: densitometry results (normalized to β-actin signal) from RT-PCR products for Y1 receptor mRNA in WKY vs SHR kidneys (left) and preglomerular microvessels (PGMV; right).
The results of the present study strongly support the concept that \( Y_1 \) receptors enhance renal vasoconstrictor responses to Ang II and that the potentiation of Ang II responses by \( Y_1 \) receptors is greater in SHR compared with WKY rats. In this regard, our results show that LPNPY, a highly selective \( Y_1 \)-receptor agonist, greatly enhances the renovascular response to Ang II in the SHR kidney, yet does not potentiate responses to Ang II in the WKY kidney. Moreover, our findings demonstrate that BIBP3226, a highly selective \( Y_1 \) receptor antagonist, blocks the ability of LPNPY to enhance renovascular responses to Ang II. This latter finding confirms that the effects of LPNPY are, indeed, mediated mostly by the \( Y_1 \) receptor.

The results of the present study also indicate that \( Y_2 \) receptors enhance (albeit only slightly) renal vasoconstrictor responses to Ang II and that the potentiation of Ang II responses by \( Y_2 \) receptors is greater in SHR compared with WKY rats. In this regard, PYY\(_{3-36}\), a highly selective \( Y_2 \)-receptor agonist, slightly enhances the renovascular response to Ang II in the SHR kidney, yet does not potentiate responses to Ang II in the WKY kidney. The observation that BIIIE0246, a highly selective \( Y_2 \)-receptor antagonist, completely abrogates the ability of PYY\(_{3-36}\) to enhance Ang II–induced renal vasoconstriction confirms that the effects of PYY\(_{3-36}\) are, indeed, mediated by the \( Y_2 \) receptor with no involvement of other PP-fold peptide receptors.

The findings of the current study have important implications regarding the pathophysiology of genetic hypertension. Renal sympathetic nerves release 2 major neurotransmitters, norepinephrine and NPY.\(^{17} \) Norepinephrine causes direct vasoconstriction by \( \alpha_1 \)-adrenoceptors that reside within the...
neuroeffector junction. Importantly, NPY can bind to and activate Y_1 and Y_2 receptors. Our findings indicate, therefore, that an important contributing cause to genetic hypertension could be corelease of NPY from renal sympathetic nerves with subsequent activation of postjunctional Y_1 receptors and, perhaps to a much lesser extent, Y_2 receptors, leading to significant potentiation of the renal vasoconstrictor responses to Ang II. The fact that acute blockade of Y_1 receptors does not lower arterial blood pressure in SHRs does not disprove this hypothesis, because only chronic treatment with a Y_1 receptor antagonist would be expected to lower arterial blood pressure in SHRs by causing a leftward shift in the renal-pressure natriuresis relationship.

It has long been known that chronic treatment of SHR with α_1-adrenoceptor blockers, for example, terazosin, does not alter the course of hypertension in SHRs. Because α_1-adrenoceptors are primarily responsible for sympathetically mediated direct vasoconstriction in the kidney, these data would appear to rule out the involvement of renal sympathetic nerves in the pathophysiology of genetic hypertension. However, chronic denervation of the SHR kidney both delays the development of hypertension and attenuates the maximum increase in blood pressure in SHRs, findings consistent with a role for the renal sympathetic nerves in hypertension, yet inconsistent with the results of studies with α_1-adrenoceptor blockers. Moreover, the profound effects of renal denervation on the natural history of hypertension in SHRs are seemingly at odds with the important role of the RAS in genetic hypertension. Our hypothesis would unify these facts by postulating that the problem is not over-activation of α_1-adrenoceptors or over-activation of the RAS, but rather normal levels of stimulation of Y_1 receptors and, perhaps to a lesser extent, Y_2 receptors, leading to potentiation of the renal vasoconstrictor responses to normal levels of Ang II in SHR, but not WKY, kidneys. It would appear that Y_1 receptors would take the leading role in this scenario, because the efficacy of Y_2 receptors with regard to potentiating Ang II–induced renal vasoconstriction seems quite low.

Why does Y_1 and Y_2 receptor activation potentiate Ang II–induced renal vasoconstriction in SHR, but not WKY, kidneys? One possibility is that Y_1 and Y_2 receptor levels are elevated in the kidneys of SHRs. However, we are unable to detect an increase in either Y_1 or Y_2 receptor mRNA or protein in either whole kidneys or preglomerular microvessels freshly isolated from whole kidneys. Thus, it appears that the mechanism for the enhanced Y_1 and Y_2 receptor activation does not involve overexpression of receptors, but rather enhanced coincidence signaling between Ang II and Y_1 and Y_2 receptor signal transduction pathways in SHR kidneys. In this regard, previous work from our laboratory indicates that the G_i pathway mediates potentiation of Ang II–induced renal vasoconstriction by α_2-adrenoceptors in SHRs. The present study shows that pertussis toxin, which ADP ribosylates and inactivates G_α, blocks the potentiation of Ang II–induced renal vasoconstriction by Y_1 and Y_2 receptors in the SHRs. Thus, like α_2-adrenoceptors, Y_1 and Y_2 receptors appear to enhance renovascular responses to Ang II in the SHRs by activating the G_i pathway. Several studies implicate overexpression of G_i proteins in some tissues in SHRs. However, our studies suggest normal levels of G_i proteins in SHR preglomerular microvessels and indicate a role for the G_i protein/phospholipase C/protein kinase Cα/ src/phosphatidylinositol 3-kinase pathway in the interaction between Ang II and the G_i signal transduction pathway in SHRs. Although we do not know why coincidence signaling between the Ang II signal transduction pathway and the G_i signal transduction pathway is enhanced in SHR kidneys, it appears to be because of a downstream event in the aforementioned signal transduction pathway. Additional studies are required to more precisely identify the involved mechanisms.

To the best of our knowledge, this study represents the first investigation of the interaction between Ang II and Y_1 and Y_2 receptors on renovascular resistance in either normotensive or hypertensive animals. However, Mohy El-Din and Malik have examined the effects of high concentrations of NPY (17 nmol/L), a potent endogenous agonist that activates nonselectively Y_1, Y_2, and Y_3 receptors, on Ang II–induced renal vasoconstriction in isolated, perfused kidneys obtained from normotensive Sprague-Dawley rats. Their studies showed that NPY at the concentration used transiently increased basal renal perfusion pressure by ∼58% and potentiated the changes in renal perfusion pressure induced by bolus injections of Ang II by ∼32%. Thus, the work by Mohy El-Din and Malik indicates that high concentrations of a “broad-spectrum” PP-fold peptide receptor agonist enhance Ang II–induced renal vasoconstriction in normotensive animals. In the present study, we did not examine the interaction between Ang II and concentrations of LPNPY >10 nmol/L, because at higher concentrations LPNPY caused a marked and sustained increase in basal renal perfusion pressure that would have confounded interpretation of the interaction between LPNPY and Ang II.

The focus of the present study was on the interaction between Y_1 and Y_2 receptors and Ang II. It is well known that in many vascular preparations, including the isolated, perfused rat kidney, NPY enhances norepinephrine-induced vasoconstriction. Whether this interaction is greater in SHR compared with WKY kidneys is an important and open question that should be addressed in future studies. Along these lines, a very recent study by Vonend et al indicates that Y_1-receptor activation enhances purinergic, nonadrenergic renal vasoconstriction in isolated, perfused kidneys from young and adult WKY and young stroke-prone SHRs but not in kidneys from adult stroke-prone SHRs. Thus, the greater enhancement of vasoconstrictor responses in SHR compared with WKY kidneys by Y_1 receptors is not due to a single mediator, but rather involves multiple mechanisms.

**Perspectives**

There are 2 known sources of endogenous agonists of renal Y_1 and Y_2 receptors. The sympathetic nervous system releases NPY at renal neuroeffector junctions, and enterocytes in the intestines release PYY_1-36 and PYY_3-36 into the circula-
tion in response to food. Thus, there is ample opportunity for endogenous agonists to activate Y₁ and Y₂ receptors. The present study demonstrates that activation of Y₁ and, to a much lesser extent, Y₂ receptors potentiates Ang II–induced renal vasoconstriction in the SHR but not the WKY. These findings indicate that, in genetically susceptible subjects, endogenous agonists of Y₁ receptors and, to a much lesser extent, Y₂ receptors, may facilitate Ang II–induced renal vasoconstriction and thereby contribute to the pathophysiology of hypertension. This work, therefore, suggests that, in genetically susceptible subjects, interactions among the RAS, renal sympathetic nervous system, and perhaps even the intestines may contribute to the pathophysiology of hypertension by vasoconstricting the renal vasculature.

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References


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