α_2-ADRENOCEPTORS POTENTIATE ANGIOTENSIN II AND VASOPRESSIN INDUCED RENAL VASOCONSTRICTION IN SHR

Liping Gao [§*] , Chongxue Zhu[§*], and Edwin K Jackson [§*‡]

[§]Center for Clinical Pharmacology, *Department of Medicine, and [‡]Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261, USA

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

Running Title: α_2-Adrenoceptor Interactions in the Kidney

Document Properties: Text pages, 30; Tables, 1; Figures, 4; References, 24; Abstract, 250 words; Introduction, 296 words; Discussion, 1500 words

Correspondence Address:

Dr. Edwin K. Jackson

Center for Clinical Pharmacology

623 Scaife Hall

University of Pittsburgh School of Medicine

3550 Terrace Street

Pittsburgh, PA 15261

Telephone: (412) 648-1880

Fax: (412) 648-7107

E-mail: edj+@pitt.edu

Abbreviations: PGMVs, preglomerular microvessels; Ang II, angiotensin II; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat; AT_1, angiotensin II type 1 receptor; V_{1a}, vasopressin type 1a receptor.

Recommended Section Assignment: Cardiovascular

ABSTRACT

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

Hypertension in spontaneously hypertensive rats (SHR) is due in part to enhanced effects of vasoactive peptides on the renal vasculature. We hypothesize that the Gi signal transduction pathway enhances renovascular responses to vasoactive peptides in SHR more so than in normotensive Wistar-Kyoto (WKY) rats. To test this hypothesis, we examined in isolated perfused kidneys from SHR and WKY rats renovascular responses (assessed as changes in renal perfusion pressure in mm Hg) to angiotensin II (10 nmoles/L) and vasopressin (3 nmoles/L) in the presence and absence of UK 14,304 (an agonist that selectively activates the Gi pathway by stimulating α_2-adrenoceptors). In SHR, but not WKY, kidneys, UK 14,304 (10 nmoles/L) enhanced ($P<0.05$) renovascular responses to angiotensin II (control WKY, 43 ± 6; UK 14,304-treated WKY, 52 ± 19; control SHR, 66 ± 17; UK 14,304-treated SHR, 125 ± 16) and vasopressin (control WKY, 42 ± 17; UK 14,304-treated WKY, 36 ± 11; control SHR, 16 ± 8; UK 14,304-treated SHR, 83 ± 17). Pretreatment of SHR with pertussis toxin (30 μg/kg, intravenously, 3-4 days before study) to inactivate Gi blocked the effects of UK 14,304. Western blot analysis of receptor expression in whole kidney and preglomerular microvessels revealed similar levels of expression of AT_1, V_{1a} and α_{2A} receptors in SHR compared with WKY rats. We conclude that activation of α_2-adrenoceptors selectively enhances renovascular responses to angiotensin II and vasopressin in SHR via an enhanced crosstalk between the Gi signal transduction pathway and signal transduction pathways activated by angiotensin II and vasopressin.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

The spontaneously hypertensive rat (SHR) is a widely employed model of human genetic hypertension; however, the pathophysiology of hypertension in SHR has eluded four decades of intensive investigation. Nonetheless, progress has been made, and it is now clear that hypertension in SHR requires the vasoactive peptide angiotensin II (Ang II) (Lee et al., 1991; Bunkenburg et al., 1991), yet SHR do not have increased circulating (Shiono and Sokabe, 1976) or tissue concentrations of Ang II (Campbell et al., 1995). It is also apparent that the SHR kidney is critical to the hypertensive state in this genetic model. In this regard, transplantation studies indicate that hypertension tracks the SHR kidney (Rettig and Unger, 1991). A third requirement for full expression of hypertension in SHR is an intact Gi signal transduction pathway. Blockade of the Gi signal transduction pathway with a single dose of pertussis toxin causes a prolonged antihypertensive response in adult SHR (Kost et al., 1999) and delays the development of hypertension in young SHR (Li and Anand-Srivastava, 2002). To understand the pathophysiology of hypertension in the SHR, the challenge is to propose and test hypotheses that reconcile these seemingly unrelated findings.

We hypothesize that hypertension in SHR is caused in part by an augmented crosstalk in the renal microcirculation between the Gi signal transduction pathway and the signal transduction pathway used by vasoactive peptides. This hypothesis predicts that activation of the Gi pathway in the renal vasculature should potentiate renovascular responses to vasoactive peptides in SHR kidneys and more so than in kidneys from normotensive Wistar-Kyoto rats (WKY). This hypothesis also predicts that inhibition of Gi with pertussis toxin should prevent potentiation of renovascular responses to vasoactive peptides by the Gi pathway in SHR kidneys. The purpose of this investigation was to test these predictions.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

METHODS

Animals. Studies utilized adult male SHR and WKY from Taconic Farms (Germantown, NY). Some SHR were anesthetized with halothane 3 to 4 days before the perfusion experiments and were administered an intravenous (via jugular) injection of 30 μg/kg of pertussis toxin. We have shown previously that this technique blocks signaling via the adenosine A_1 receptor, a Gi-coupled receptor, in the kidney (Kost et al., 2000). The Institutional Animal Care and Use Committee approved all procedures.

Method of Isolated Perfused Rat Kidney. Rats were anesthetized with Inactin (100 mg/kg, i.p.), the left ureter was cannulated with polyethylene (PE)-10 tubing, and the left inferior suprarenal artery was ligated and severed. The aorta was cleared above and below the left renal artery, and two silk ties were placed loosely above (ties A and B) and below (ties C and D) the left renal artery. Ties B and C were positioned just above and below the left renal artery, respectively; ties A and D were positioned approximately one cm above and below the left renal artery, respectively. Two silk ties (E and F) were also placed loosely around the left renal vein. In this regard, tie E was positioned near the inferior vena cava and tie F was placed near the renal hilus.

Once all ties were in place, tie D on the lower aorta was tightened, and a small vascular clamp was placed on the aorta just rostral to tie C. An incision was made in the aorta just rostral to tie D, and a PE-50 cannula connected to the perfusion system was inserted into the aorta. This cannula was advanced to the vascular clamp and secured in place with tie C. The vascular clamp was removed, the perfusion pump (see below) was activated to begin perfusion with Tyrode's solution, and ties A and B on the aorta above the renal artery were secured. Tie E on the renal vein near the inferior vena cava

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

was secured, and an incision was made in the renal vein. A catheter was inserted into the renal vein and secured in place with tie F. The perfusion catheter in the aorta was advanced into the renal artery and secured with an additional tie 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 to a Hugo Sachs Elektronik-Harvard Apparatus GmbH (March-Hugstetten, Germany) kidney perfusion system. This system included the following components: a Model UP 100 Universal Perfusion System, a Model ISM 834 Channel Reglo Digital Roller Pump, a glass double-walled perfusate reservoir, a R 120144 glass-oxygenator that was mechanically integrated with the Model UP 100 Universal Perfusion System, a Windkessel for absorption of pulsations, an inline holder for disc particle filters (80 microns), a temperature controlled plexiglass kidney chamber integrated with the UP 100 Universal Perfusion System, 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 (NaCl, 137 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM; $MgCl_2$, 1.1 mM; $NaHCO_3$, 12 mM; NaH_2PO_4, 0.42 mM; D(+)-glucose, 5.6 mM; pH, 7.4) was maintained at 37°C in the double-walled perfusate reservoir and was bubbled with 95% oxygen/5% carbon dioxide. The warmed and oxygenated Tyrode's solution was pumped by the roller pump to a glass oxygenator (95% oxygen/5% carbon dioxide), then through an inline particle filter, then through an inline Windkessel, then through a heat exchanger, then 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 with a Statham pressure transducer (model P23ID; Statham Division, Gould Inc., Oxnard,

CA) and recorded on a Grass model 79D polygraph (Grass Instruments, Quincy, MA).

Protocol with Isolated Perfused Rat Kidney. After at least a 30-minute rest period, a vasoconstrictor response (change in perfusion pressure) to 10 nmoles/L of Ang II was elicited by infusing Ang II for 2 minutes. After another rest period during which baseline perfusion pressure had returned to normal, a vasoconstrictor response to 3 nmoles/L of vasopressin was elicited by infusing vasopressin for 2 minutes. These experiments were performed in five groups of kidneys as follows: 1) control SHR, n=6; 2) control WKY, n=6; 3) UK 14,304-treated SHR, n=6; 4) UK 14,304-treated WKY, n=6; 5) pertussis toxin-pretreated and UK 14,304-treated SHR, n=6. Treatment with UK 14,304 was begun 5 to 10 minutes before each response to Ang II or vasopressin and was continued for the 2-minute infusion of Ang II or vasopressin.

Isolation of Preglomerular Microvessels. SHR and WKY rats were anesthetized with Inactin (100 mg/kg, i.p.), and the kidneys were flushed with room temperature L-15 medium (Leibovitz medium; Sigma Co., St. Louis, MO). A 1% suspension of iron oxide (Aldrich Chemical Co., Milwaukee, WI) in L-15 medium (10 ml; 37°C) was flushed into the kidneys. The kidneys were placed in ice-cold L-15 medium, and the cortex was obtained, minced, suspended in ice-cold L-15 medium and dispersed by pushing the cortical material through a series of needle hubs (3 times through 16, 18, and 21 gauge and 6 times through 23 gauge). The cortical material was suspended in ice-cold L-15 medium and a magnet was applied to the tube to retrieve the iron oxide laden preglomerular microvessels (PGMVs) while the unwanted material was decanted. The glomeruli were removed from the microvessels by filtering the suspension of PGMVs through a 149-micron nylon mesh. The PGMVs were retrieved from the nylon mesh and placed in ice-cold phosphate-buffered (PBS) saline.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

Protein Extraction. PGMVs were frozen in liquid nitrogen and ground into a powder on liquid nitrogen. The ground tissues were placed in a tube with 0.5 ml SDS buffer (50 mM Tris, pH 7.0; 2% SDS; 10% glycerol) containing protease inhibitors (antipain, 2 μg/ml; aprotinin, 1 μg/ml; leupeptin, 2 μg/ml; 1 mg/ml PMSF) and homogenized. The homogenate was centrifuged at 12,000 rpm at 4°C for 10 minutes, and the supernatant was recovered. Protein in the supernatant was determined by the copper bicinchoninic acid method, and samples were stored at –20°C.

Western Blotting. Laemmli buffer was added to samples, and samples were placed in boiling water for 5 minutes and then chilled on ice. Samples (30 μg protein in each well) were loaded onto a 7.5% acrylamide gel and subjected to SDS-PAGE using the Bio-Rad mini-gel system. Proteins were then electroblotted onto a PVDF membrane (Millipore, Bedford, MA). The PVDF membrane was blocked with 5% milk for 1 hour and incubated for 3 hours at room temperature with the primary antibody. We used one of four different primary antibodies, either anti-AT_1 receptor antibody (Cat. No. sc-1173, Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-AT_2 receptor antibody Cat. No. RDI-ANG1O2R20br, Research Diagnostics, Inc., Flanders, NJ), anti-α_{2A}-adrenoceptor antibody (Cat. No. A269, Sigma) or anti-V_{1a} receptor antibody (RDI-AVPRV1AXabr, Research Diagnostics, Inc. Flanders, NJ). Primary antibodies were diluted 1:1000 in PBS containing 0.05% Tween-20. Membranes were washed in PBS containing 0.05% Tween-20 solution three times and then incubated at room temperature for 1 hour with horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody (Amersham, Arlington Heights, IL) at 1:5,000 dilution. Membranes were exposed to films and the signals were detected by Supersignal Substrate kit (Pierce, Rockford, IL). Band densities were quantitatively measured using Scion-image software. Background signals were obtained in each lane and subtracted from the band densities to correct for the background signal.

Statistical Analysis. A global analysis of the data was performed by 3-factor analysis of variance in which factor A was rat strain (WKY versus SHR), factor B was level of treatment with UK 14,304 (0 or 10 nmoles/L) and factor C was peptide agonist (Ang II or vasopressin). Additional comparisons were performed by 1-factor analysis of variance. Post hoc comparisons were performed using the Fisher's Least Significant Difference test. The criterion of significance was $P<0.05$, and all data are expressed as the mean ± SEM.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

RESULTS

As shown in Table 1, baseline perfusion pressures in the SHR control group were slightly, yet significantly ($P<0.05$), greater compared with the WKY control group. However, baseline perfusion pressures were not affected significantly by UK 14, 304 in either WKY or SHR, and baseline perfusion pressures were not significantly different in the SHR UK 14,304 group compared with the WKY UK 14,304 group. Pertussis toxin did not affect baseline perfusion pressure in SHR (Table 1).

As illustrated in Figure 1 (panel A) the renovascular response to Ang II (10 nmoles/L) was not significantly different in control WKY kidneys versus WKY kidneys pretreated with UK 14,304 (10 nmoles/L). In contrast in SHR kidneys, UK 14,304 (10 nmoles/L) significantly increased vasoconstrictor responses to Ang II (10 nmoles/L) by approximately two-fold (Figure 1, panel B). However, in kidneys removed from SHR pretreated three to four days before the experiment with pertussis toxin (30 μg/kg, intravenously), UK 14,304 did not alter Ang II-induced renovascular responses (Figure 1, panel B). Renovascular responses to Ang II in the absence of UK 14,304 were not significantly different in WKY versus SHR kidneys (Figure 1, panel A versus panel B); however in the presence of UK 14,304, renovascular responses to Ang II were significantly greater in SHR compared with WKY kidneys (Figure 1, panel A versus panel B).

As illustrated in Figure 2 (panel A) the renovascular response to vasopressin (3 nmoles/L) was not significantly different in control WKY kidneys versus WKY kidneys pretreated with UK 14,304 (10 nmoles/L). In contrast in SHR kidneys, UK 14,304 (10 nmoles/L) significantly increased vasoconstrictor responses to vasopressin (3 nmoles/L) by approximately five-fold (Figure 2, panel B). However, in kidneys removed from SHR pretreated three to four days before the experiment with

pertussis toxin (30 μg/kg, intravenously), UK 14,304 did not alter vasopressin-induced renovascular responses (Figure 2, panel B). Renovascular responses to vasopressin in the absence of UK 14,304 were not significantly different in WKY versus SHR kidneys (Figure 2, panel A versus panel B); however in the presence of UK 14,304, renovascular responses to vasopressin were nearly significantly greater ($P < 0.1$) in SHR compared with WKY kidneys (Figure 2, panel A versus panel B).

A 3-factor analysis of variance was performed to examine more completely the interaction between rat strain and the effects of UK 14,304 on renovascular responses to Ang II and vasopressin. In this analysis, the factors were rat strain (WKY versus SHR), level of UK 14,304 (0 versus 10 nmoles/L) and the vasoactive peptide (Ang II versus vasopressin). This analysis revealed a significant ($P=0.016$) interaction between rat strain and UK 14,304 on renovascular responses to the vasoactive peptides; however the interaction between rat strain and UK 14,304 was independent of the type of vasoactive peptide ($P=0.801$ for 3-way interaction between strain, UK 14,304 and vasoactive peptide).

Western blot analysis of PGMVs or whole kidney using the anti-AT_1 receptor antibody gave rise to a clear and prominent band at 41.7 kDa, which is approximately the nominal mass (41 kDa) of the AT_1 receptor. Expression of the AT_1 receptor was greater in PGMVs (Figure 3, panel A) compared with whole kidney (Figure 4, panel A); however, expression of the AT_1 receptor was not different in WKY versus SHR either in PGMVs (Figure 3, panel A) or whole kidney (Figure 4, panel A). AT_2 receptor expression could not be detected by Western blotting or by RT-PCR followed by ethidium bromide staining in either whole kidneys or freshly isolated PGMVs in either SHR or WKY (data not shown).

Western blot analysis of PGMVs or whole kidney using the anti-V_{1a} receptor antibody gave

rise to a clear and prominent band at 46 kDa, which is approximately the nominal mass (44 kDa) of the V_{1a} receptor. Expression of the V_{1a} receptors was greater in PGMVs (Figure 3, panel B) compared with whole kidney (Figure 4, panel B); however, expression of the V_{1a} receptor was not different in WKY versus SHR either in PGMVs (Figure 3, panel B) or whole kidney (Figure 4, panel B).

Western blot analysis of PGMVs and whole kidney using the anti-α_{2A}-adrenoceptor antibody gave rise to a clear and dominant band at 54 kDa, which is slightly larger than the nominal mass (49 kDa) of the α_{2A}-adrenoceptor and may represent a glycosylated and/or palmitoylated form of the receptor. Expression of the α_{2A}-adrenoceptor was greater in PGMVs (Figure 3, panel C) compared with whole kidney (Figure 4, panel C); however, expression of the α_{2A}-adrenoceptor was not different in WKY versus SHR either in PGMVs (Figure 3, panel C) or whole kidney (Figure 4, panel C).

DISCUSSION

In the SHR, hypertension tracts the kidney (Rettig and Unger, 1991) and requires an intact renin-angiotensin system (Lee et al., 1991; Bunkenburg et al., 1991); however, Ang II production is not excessive in SHR (Shiono and Sokabe, 1976; Campbell et al., 1995). Previous studies indicate that these facts can be explained by an enhanced renovascular sensitivity to Ang II *in vivo* (Li and Jackson, 1989; Chatziantoniou et al., 1990; Chatziantoniou and Arendshorst, 1991; Kost and Jackson, 1993). The mechanism of this enhanced renal sensitivity is under intense investigation.

In a recent *in vivo* study (Jackson et al., 2001), we measured increases in renovascular resistance in response to intrarenal infusions of Ang II and vasopressin in the presence and absence of co-activation of α_2-adrenoceptors (i.e., receptors selectively coupled to Gi) with UK 14,304 in adrenalectomized, renal-denervated, captopril-pretreated SHR and WKY rats. In that study, we observed that in SHR, but not WKY rats, UK 14,304 markedly enhanced renovascular responses to Ang II and vasopressin. We also observed that UK 14,304 did not enhance renovascular responses to methoxamine (α_1-adrenoceptor agonist) in either strain. Moreover, we observed that in rats with non-genetic hypertension, UK 14,304 had little effect on renovascular responses to Ang II. We concluded that activation of α_2-adrenoceptors selectively enhances renovascular responses to Ang II and vasopressin *in vivo* in animals with genetic hypertensive, but not in normotensive animals or animals with acquired hypertension.

Our previous results suggest that in SHR there is a genetically-mediated enhanced crosstalk between the Gi signal transduction pathway and signal transduction pathways activated by angiotensin

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

II and vasopressin, but not methoxamine. To further test our hypothesis, in subsequent experiments we attempted to examine the interaction between renal α_2-adrenoceptors and Ang II or vasopressin *in vivo* in SHR pretreated with pertussis toxin to inhibit the Gi signal transduction pathway. However, we could not perform these experiments because pertussis toxin pretreated rats could not tolerate the extensive manipulations (adrenalectomy, renal denervation and captopril-pretreatment) necessary to examine this interaction *in vivo.*

One goal of the present study was to overcome the limitations of our *in vivo* paradigm by developing an *in vitro* model system that would recapitulate the *in vivo* enhanced interaction between Ang II or vasopressin and α_2-adrenoceptors. Importantly, the results of the present study demonstrate that in the isolated, perfused SHR kidney UK 14,304, a selective α_2-adrenoceptor agonist, markedly augments renovascular responses to both Ang II and vasopressin. Our results also establish that in contrast to SHR, UK 14,304 has little effect on renovascular responses to Ang II or vasopressin in the isolated, perfused WKY kidney. These results are important for two reasons. First, the present data confirm that our *in vivo* findings were not an artifact caused by uncontrolled variables in the *in vivo* experiments. Second, the present findings provide a useful *in vitro* model system in which to investigate the mechanism of the enhanced interaction between Ang II or vasopressin and α_2-adrenoceptors in the SHR kidney.

A second goal of the present study was to test the hypothesis that the interaction between Ang II or vasopressin and α_2-adrenoceptors in SHR kidneys is mediated by the Gi signal transduction pathway. In this regard, we utilized the technique of administering an intravenous bolus dose of pertussis toxin several days before the experiment (Kost et al., 2000). Pertussis toxin is well-known to

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

ADP ribosylate Gi and thereby inactivate this key signal transduction pathway. Importantly, the present study demonstrates that the ability of UK 14,304 to potentiate Ang II-mediated or vasopressin-mediated renal vasoconstriction in SHR kidneys is blocked by inhibition of the Gi signal transduction pathway with pertussis toxin.

In vivo SHR kidneys are more responsive to Ang II (Li and Jackson, 1989; Chatziantoniou et al., 1990; Chatziantoniou and Arendshorst, 1991; Kost and Jackson, 1993) and vasopressin (Feng and Arendshorst, 1996; Feng and Arendshorst, 1997) compared to WKY kidneys, and the results of the present study offer an explanation for this consistent finding. In this regard, the present findings strongly support the conclusion that in SHR kidneys there is an enhanced interaction between Ang II or vasopressin and the Gi signal transduction pathway. This conclusion is consistent with our previous reports that *in vivo* pertussis toxin normalizes augmented renovascular responses to Ang II in SHR (Jackson, 1994; Jackson et al., 1999). This conclusion is also consistent with the observation in the present study that in isolated perfused SHR kidneys in the absence of UK 14,304, renovascular responses to Ang II and vasopressin are not augmented compared with WKY kidneys. In contrast, in the presence of UK 14,304 to stimulate the Gi signal transduction pathway, renovascular responses to both Ang II and vasopressin become greater compared with control or UK 14,304-treated WKY kidneys. Evidently, *in vitro* in the absence of UK 14,304 there is insufficient activation of the Gi signal transduction pathway to render the SHR kidney hyper-responsive to Ang II and vasopressin, whereas *in vivo* there is sufficient hormonal stimulation of the Gi pathway (for example via norepinephrine, adenosine and/or neuropeptide Y acting on α_2-adrenoceptors, A_1 receptors and Y_1 receptors, respectively) to ensure hyper-responsiveness of SHR kidneys to Ang II and vasopressin. Importantly, the *in vivo* situation is reconstituted *in vitro* by the addition of an activator of the Gi signal transduction pathway.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

The observations that blockade of the Gi signal transduction pathway with a single dose of pertussis toxin causes a prolonged antihypertensive response in adult SHR (Kost et al., 1999) and delays the development of hypertension in young SHR (Li and Anand-Srivastava, 2002) indicate that the enhanced interaction between the Gi signal transduction pathway and vasoactive peptides importantly contributes to the pathophysiology of hypertension in SHR.

It is possible that the enhanced interaction between Ang II or vasopressin and α_2-adrenoceptors in the SHR kidney is merely due to increased expression of receptors for Ang II, vasopressin or UK 14,304. Ang II, vasopressin and UK 14,304 cause vasoconstriction via AT_1 receptors (Kost and Jackson, 1993), V_{1a} receptors (Feng and Arendshorst, 1996) and α_{2A}-adrenoceptors (Gavin and Docherty, 1996), respectively. The present study demonstrates that neither AT_1 receptors nor V_{1a} receptors nor α_{2A}-adrenoceptors are over-expressed in either the preglomerular microcirculation or in total kidney tissue of SHR. Moreover, AT_2 receptor expression was undetectable in either whole kidneys or PGMVs from either SHR or WKY. These results suggest that in the SHR the enhanced crosstalk between the Gi signal transduction pathway and signal transduction pathways activated by angiotensin II and vasopressin is not due to changes in receptor expression.

Vagnes and colleagues (Vagnes et al., 2000) observed in SHR PGMVs a two-fold increase in V_{1a} receptor mRNA expression by RT-PCR and a two to three times increase in V_{1a} receptor density by ligand binding. There are several key differences between our study and the study by Vagnes et al. that could explain the disparate results. These differences include age of animals (adult animals versus young animals, respectively), strain of animals (Taconic Farms versus Chapel Hill breeding colony, respectively) and technique of isolating PGMVs (no collagenase digestion versus collagenase

digestion, respectively). The difference in results between the two studies invites further exploration of the factors that determine V_{1a} receptor expression.

What is the mechanism by which the Gi signal transduction pathway participates in the enhanced interaction between α_2-adrenoceptors and Ang II or vasopressin in SHR? Both AT_1 receptors (Sano et al., 1997) and V_1 receptors (Thibonnier et al., 1993) are G_q-coupled receptors, albeit non-selectively, and Ang II and vasopressin cause renal vasoconstriction by activating AT_1 (Kost et al., 1993) and V_1 receptors (Feng and Arendshorst, 1996), respectively. Therefore, one interpretation of the present findings is that there is an enhanced ability the Gi signal transduction pathway to augment renovascular responses to G_q-coupled receptors in SHR. In this regard, it is well-established that even though $\beta\gamma$ subunits per se have no effect on phospholipase C-β, the ability of α_q to activate phospholipase C-β is markedly enhanced by $\beta\gamma$ subunits released from Gi proteins (Selbie and Hill, 1998). These considerations suggest that the mechanism by which Gi coupled receptors enhance the renovascular response to Ang II and vasopressin could involve enhanced coincident signaling between α_q and $\beta\gamma$ at the level of phospholipase C-β.

We have recently shown that phospholipase D2 is activated by Ang II in preglomerular microvascular smooth muscle cells, and this response is markedly enhanced in SHR versus WKY cells and is mediated by RhoA (Andresen et al., 2001). Moreover, recent studies show that α_2-adrenoceptors activate RhoA (Jinsi-Parimoo and Deth, 1997). Therefore, it is conceivable that the interaction between Ang II or vasopressin and α_2-adrenoceptors is mediated by coincident signaling between Ang II and vasopressin and the Gi signal transduction pathway at the level of a Rho GEF.

In conclusion, the present investigation demonstrates an augmented crosstalk in the SHR renal microcirculation between the Gi signal transduction pathway and the signal transduction pathway used by vasoactive peptides. Consequently, drugs that interfere with the Gi signal transduction pathway may offer a novel approach for the treatment of high blood pressure in human beings.

REFERENCES

Andresen BT, Jackson EK and Romero GG (2001) Angiotensin II signals to phospholipase D in the spontaneously hypertensive and Wistar-Kyoto rats by pharmacologically distinct mechanisms. *Hypertension* **37**:635-639.

Bunkenburg B, Schnell C, Baum HP, Cumin F and Wood JM (1991) Prolonged angiotensin II antagonism in spontaneously hypertensive rats: Hemodynamic and biochemical consequences. *Hypertension* **18**:278-288.

Campbell DJ, Duncan AM, Kladis A and Harrap SB (1995) Angiotensin peptides in spontaneously hypertensive and normotensive Donryu rats. *Hypertension* **25**:928-934.

Chatziantoniou C and Arendshorst WJ (1991) Angiotensin and thromboxane in genetically hypertensive rats: renal blood flow and receptor studies. *Am J Physiol* **261**:F238-F247.

Chatziantoniou C, Daniels FH and Arendshorst WJ (1990) Exaggerated renal vascular reactivity to angiotensin and thromboxane in young genetically hypertensive rats. *Am J Physiol* **259**: 372-F382.

Feng, J.J., and Arendshorst, W.J. 1996. Enhanced renal vasoconstriction induced by vasopressin in SHR is mediated by V_1 receptors. *Am J Physiol* 271:F304-F313.

Feng JJ and Arendshorst WJ (1997) Calcium signaling mechanisms in renal vascular responses to vasopressin in genetic hypertension. *Hypertension* **30**:1223-1231.

Gavin K and Docherty JR (1996) Investigation of the subtype of alpha 2-adrenoceptor mediating pressor responses in the pithed rat. *Eur J Pharmacol* **318**:81-87.

Jackson EK (1994) Pertussis toxin normalizes enhanced renovascular responses to angiotensin II in spontaneously hypertensive rats. *Life Sci* 54:PL445-PL450.

Jackson EK, Herzer WA, Kost CK Jr and Vyas SJ (2001) Enhanced interaction between renovascular α_2-adrenoceptors and angiotensin II receptors in genetic hypertension. *Hypertension* **38**:353-360.

Jackson EK, Herzer WA, Vyas SJ and Kost CK Jr (1999) Angiotensin II-induced renal vasoconstriction in genetic hypertension. *J Pharmacol Exp Ther* **291**:329-334.

Jinsi-Parimoo A and Deth RC (1997) Reconstitution of alpha2D-adrenergic receptor coupling to phospholipase D in a PC12 cell lysate. *J Biol Chem* **272**:14556-14561.

Kost CK Jr, Herzer WA, Li PJ and Jackson EK (1999) Pertussis toxin-sensitive G-proteins and regulation of blood pressure in the spontaneously hypertensive rat. *Clin Exp Pharmacol Physiol* **26**:449-255.

Kost CK Jr, Herzer WA, Rominski BR, Mi Z and Jackson EK (2000) Diuretic response to adenosine A_1 receptor blockade in normotensive and spontaneously hypertensive rats: Role of pertussis toxin-sensitive G-proteins. *J Pharmacol Exp Ther* **292**:752-760.

Kost CK Jr and Jackson EK (1993) Enhanced renal angiotensin II subtype 1 receptor responses in the spontaneously hypertensive rat. *Hypertension* **21**:420-431.

Lee RM, Berecek KH, Tsoporis J, McKenzie R and Triggle CR (1991) Prevention of hypertension and vascular changes by captopril treatment. *Hypertension* **17**:141-150.

Li Y and Anand-Srivastava MB (2002) Inactivation of enhanced expression of G_i proteins by pertussis toxin attenuates the development of high blood pressure in spontaneously hypertensive rats. *Circ Res* **91**:247-254.

Li P and Jackson EK (1989) Enhanced slow-pressor response to angiotensin II in spontaneously hypertensive rats. *J Pharmacol Exp Ther* **251**:909-921.

Rettig R and Unger T (1991) The role of the kidney in the aetiology of hypertension: renal transplantation studies in rats. *Trends Pharmacol Sci* **12**:243-245.

Sano T, Ohyama K, Yamano Y, Nakagomi Y, Nakazawa S, Kikyo M, Shirai H, Blank JS, Exton JH and Inagami T (1997) A domain for G protein coupling in carboxyl-terminal tail of rat angiotensin II receptor type 1A. *J Biol Chem* **272**:23631-23636.

Selbie LA and Hill SJ (1998) G protein-coupled-receptor cross-talk: the fine-tuning of multiple receptor-signaling pathways. *TiPS* **19**:87-93.

Shiono K and Sokabe H (1976) Renin-angiotensin system in spontaneously hypertensive rats. *Am J Physiol* **231**:1295-1299.

Thibonnier M, Goraya T and Berti-Mattera L (1993) G protein coupling of human platelet V_1 vascular vasopressin receptors. *Am J Physiol* **264**:C1336-C1344.

Vagnes O, Feng JJ, Iversen BM and Arendshorst WJ (2000) Upregulation of V_1 receptors in renal resistance vessels of rats developing genetic hypertension. *Am J Physiol* **278**:F940-F048.

FOOTNOTE

This study was supported by National Institutes of Health grant HL69846.

FIGURE LEGENDS

Figure 1: Changes in perfusion pressure induced by angiotensin II (10 nmoles/L) in control WKY kidneys (panel A), UK 14,304 (10 nmoles/L)-treated WKY kidneys (panel A), control SHR kidneys (panel B), UK 14,304-treated SHR kidneys (panel B) and UK 14,304-treated kidneys removed from SHR pretreated 3 to 4 days earlier with 30 μg/kg of pertussis toxin (panel B). Values represent means ± SEM from six animal.

Figure 2: Changes in perfusion pressure induced by vasopressin (3 nmoles/L) in control WKY kidneys (panel A), UK 14,304 (10 nmoles/L)-treated WKY kidneys (panel A), control SHR kidneys (panel B), UK 14,304-treated SHR kidneys (panel B) and UK 14,304-treated kidneys removed from SHR pretreated 3 to 4 days earlier intravenously with 30 μg/kg of pertussis toxin (panel B). Values represent means ± SEM from six animals.

Figure 3: Bar graphs illustrating the expression of AT_1 receptors, V_{1A} receptors and α_{2A}-adrenoceptors in freshly isolated preglomerular microvessels (PGMVs) in SHR and WKY rats. Samples (30 μg protein in each well) were loaded onto a 7.5% acrylamide gel and subjected to SDS-PAGE using the Bio-Rad mini-gel system. Proteins were then electroblotted onto a PVDF membrane. The PVDF membrane was blocked with 5% milk for 1 hour and incubated for 3 hours at room temperature with the primary antibody (anti-AT_1 receptor, anti-V_{1A} receptors or anti-α_{2A}-adrenoceptor). Membranes were washed in PBS containing 0.5% Tween-20 solution three times and then incubated at room temperature for 1 hour with horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody. Membranes were exposed to films and the signals were detected by Supersignal Substrate kit. Band densities were quantitatively measured using Scion-image software.

Background signals were obtained in each lane and subtracted from the band densities to correct for the background signal. Values represent means ± SEM for three observations.

Figure 4: Bar graphs illustrating the expression of AT_1 receptors, V_{1A} receptors and α_{2A}-adrenoceptors in whole kidneys from SHR and WKY rats. Samples (30 µg protein in each well) were loaded onto a 7.5% acrylamide gel and subjected to SDS-PAGE using the Bio-Rad mini-gel system. Proteins were then electroblotted onto a PVDF membrane. The PVDF membrane was blocked with 5% milk for 1 hour and incubated for 3 hours at room temperature with the primary antibody (anti-AT_1 receptor, anti-V_{1A} receptors or anti-α_{2A}-adrenoceptor). Membranes were washed in PBS containing 0.5% Tween-20 solution three times and then incubated at room temperature for 1 hour with horseradish peroxidase-conjugated donkey anti-rabbit IgG secondary antibody. Membranes were exposed to films and the signals were detected by Supersignal Substrate kit. Band densities were quantitatively measured using Scion-image software. Background signals were obtained in each lane and subtracted from the band densities to correct for the background signal. Values represent means ± SEM for three observations.

JPET Fast Forward. Published on February 11, 2003 as DOI: 10.1124/jpet.102.047647
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1: Baseline perfusion pressures (mm Hg) just before administration of angiotensin II or vasopressin.

		WKY	SHR
BEFORE ANGIOTENSIN II			
	CONTROL	37 ± 1.6	46 ± 2.8[a]
	UK 14,304	36 ± 2.2	43 ± 3.1
	UK 14,304 + Pertussis Toxin		42 ± 2.5
BEFORE VASOPRESSIN			
	CONTROL	38 ± 2.3	50 ± 3.5[a]
	UK 14,304	37 ± 2.3	44 ± 2.7
	UK 14,304 + Pertussis Toxin		42 ± 2.5

[a]P<0.05, SHR control versus WKY Control. Values are means ± SEM for six observations.

Figure 1

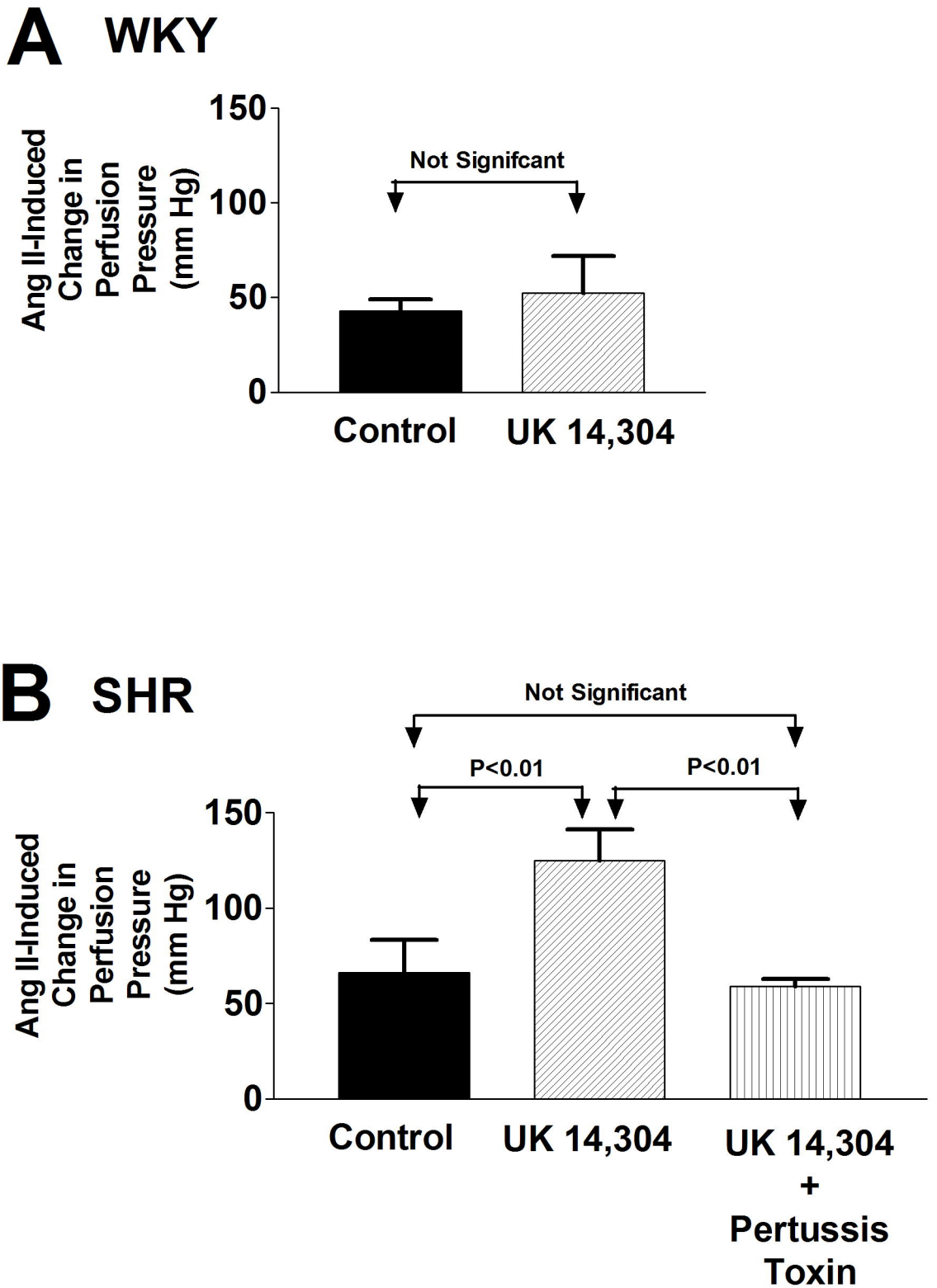

A
WKY
Ang II-Induced Change in Perfusion Pressure (mm Hg)
150
100
50
0
Not Signifcant
Control
UK 14,304
B
SHR
Not Significant
P<0.01
P<0.01
Ang II-Induced Change in Perfusion Pressure (mm Hg)
150
100
50
0
Control
UK 14,304
UK 14,304 + Pertussis Toxin

Figure 2

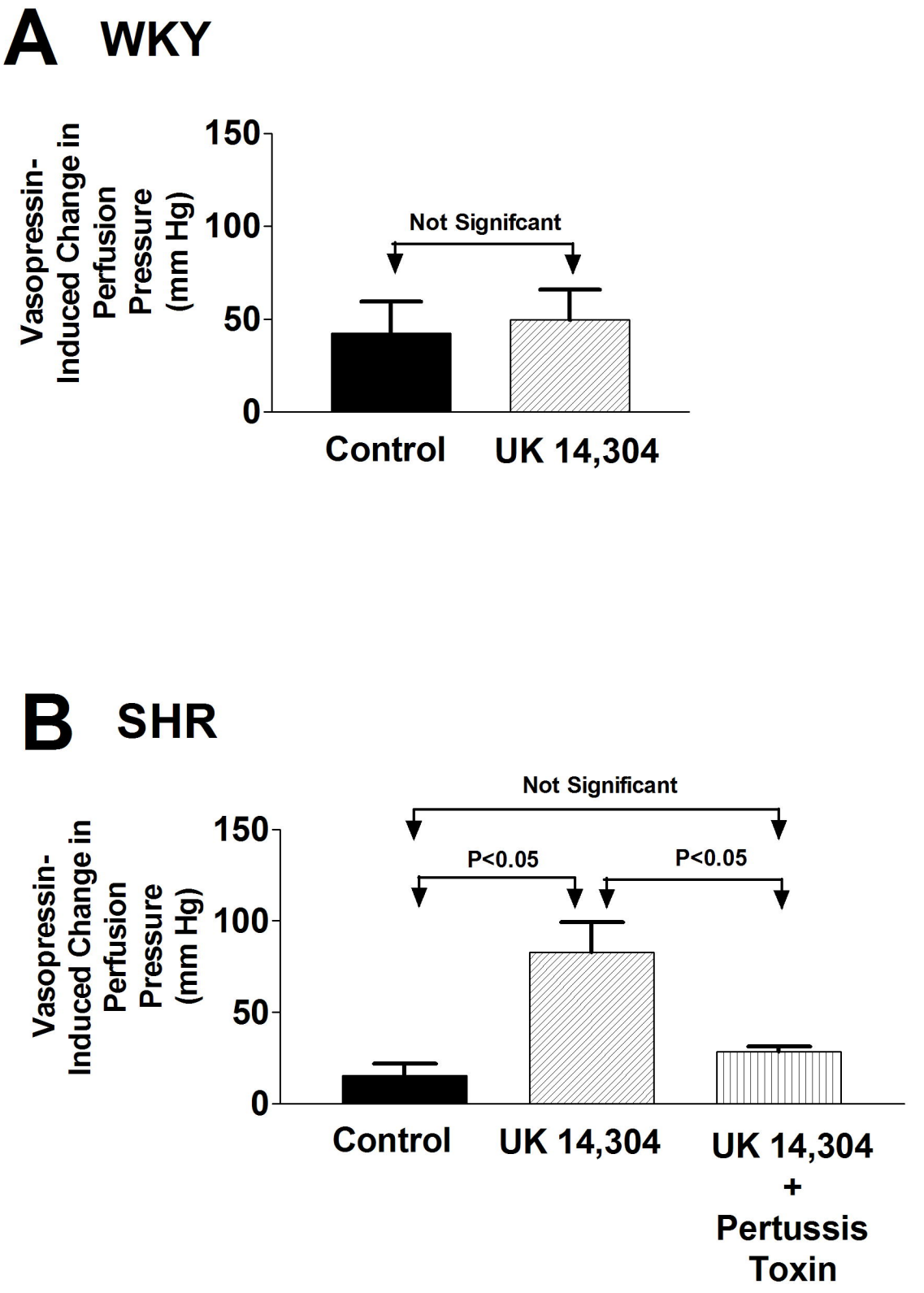

A
WKY
Vasopressin-Induced Change in Perfusion Pressure (mm Hg)
150
100
50
0
Not Signifcant
Control
UK 14,304
B
SHR
Vasopressin-Induced Change in Perfusion Pressure (mm Hg)
150
100
50
0
Not Significant
P<0.05
P<0.05
Control
UK 14,304
UK 14,304 + Pertussis Toxin

Figure 3

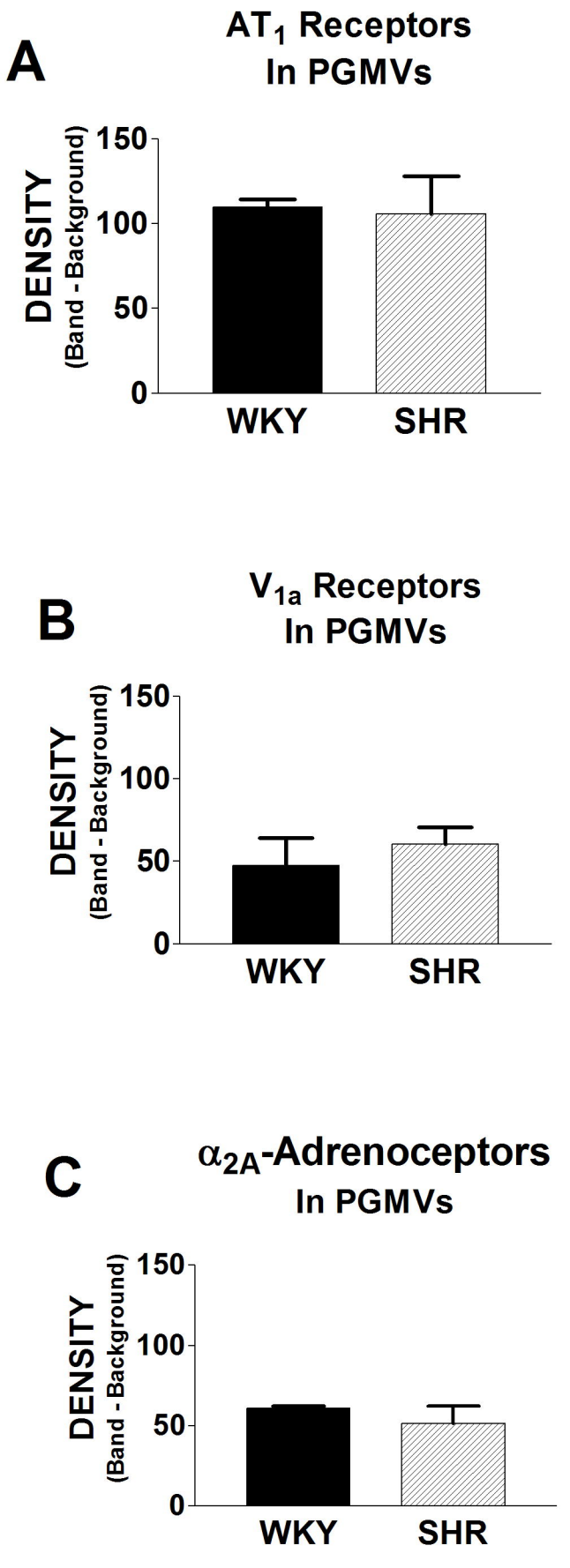
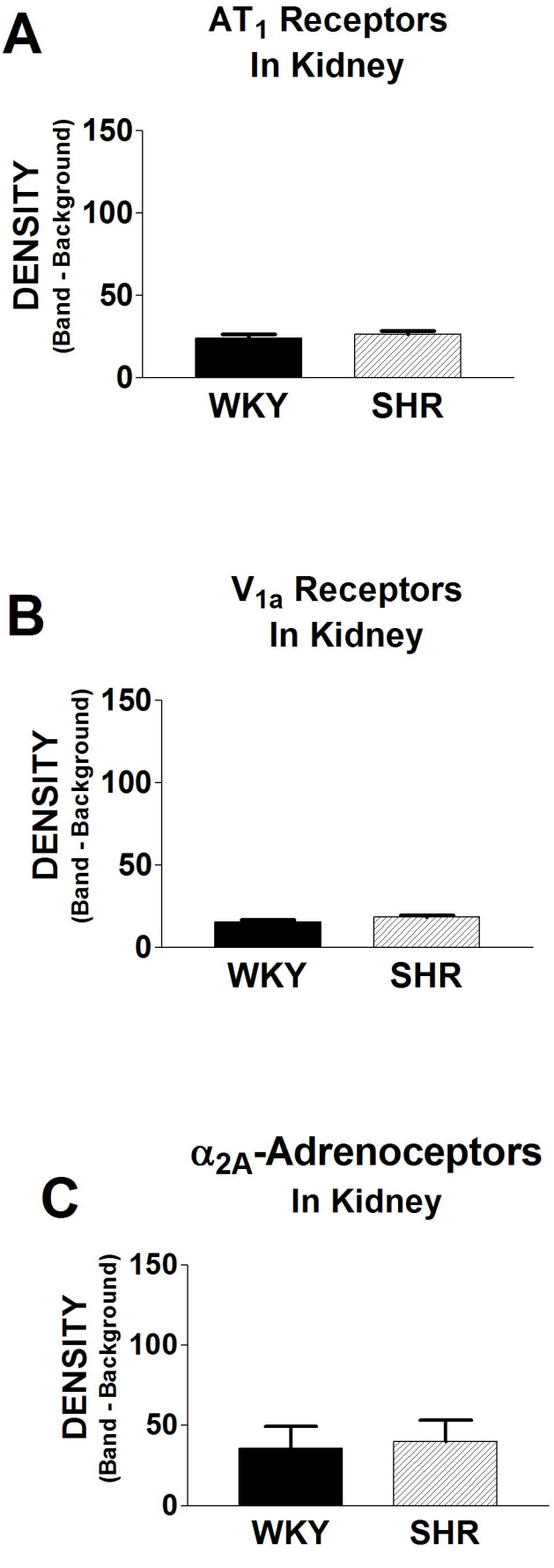

A
AT1 Receptors
In PGMVs
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR
B
V1a Receptors
In PGMVs
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR
C
α2A-Adrenoceptors
In PGMVs
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR

Figure 4


A
AT1 Receptors
In Kidney
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR
B
V1a Receptors
In Kidney
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR
C
α2A-Adrenoceptors
In Kidney
DENSITY
(Band - Background)
150
100
50
0
WKY
SHR