Impaired Interaction Between Efferent and Afferent Renal Nerve Activity in SHR Involves Increased Activation of $\alpha_2$-Adrenoceptors

Ulla C. Kopp, Michael Z. Cicha, Lori A. Smith

Abstract—Activation of efferent renal sympathetic nerve activity (ERSNA) increases afferent renal nerve activity (ARNA), leading to decreases in ERSNA by activation of the renorenal reflexes in the overall goal of maintaining low ERSNA. The renorenal reflex responses to various stimuli are impaired in spontaneously hypertensive rats (SHR). Because renal tissue density of $\alpha_2$-adrenoceptors (ARs) is increased in SHR, we examined whether the ERSNA-induced increases in ARNA are impaired in SHR and, if so, the role of $\alpha_2$-ARs. The ARNA responses to increases in ERSNA were impaired in SHR, 2390±460%-seconds, versus in Wistar-Kyoto rats, 6620±1690%-seconds. Renal pelvic release of substance P was not altered by 6250 pmol/L norepinephrine (NE) in SHR but was increased by 250 pmol/L NE in Wistar-Kyoto rats, from 5.7±0.7 to 12.5±1.3 pg/min. Renal pelvic administration of the $\alpha_2$-AR antagonist rauwolscine enhanced the ERSNA-induced increases in ARNA, 4170±900%-seconds, in SHR but not in Wistar-Kyoto rats. In the presence of rauwolscine, 250 pmol/L NE increased substance P release, from 5.2±0.3 to 11.2±0.8 pg/min, in pelvises from SHR. Because angiotensin II suppresses the activation of renal mechanosensory nerves in SHR, we examined whether losartan improved the ERSNA-induced ARNA responses. Losartan had no effect on the ARNA responses or the NE-induced increases in substance P in SHR. However, losartan+rauwolscine resulted in further enhancement of the responsiveness of the renal sensory nerves to increases in ERSNA and NE in SHR but not in WKY. We conclude that increased activation of renal $\alpha_2$-ARs and angiotensin II type 1 receptors contributes to the impaired interaction between ERSNA and ARNA in SHR. (Hypertension. 2011;57[part 2]:640-647.) • Online Data Supplement

Key Words: kidney • sensory nerves • sympathetic nerves • substance P • PGE2 • angiotensin

The kidney has a rich supply of sympathetic nerves that innervate all parts of the nephron and the renal vasculature.1 The kidney also has abundant afferent sensory innervation, located primarily in the renal pelvic wall.2,3 Sympathetic efferent nerve fibers and afferent sensory nerve fibers often run separately but intertwined in the same nerve bundles in the renal pelvic wall,2 providing anatomic support for a functional interaction between efferent renal sympathetic nerve activity (ERSNA) and afferent renal nerve activity (ARNA). In normotensive rats, activation of the renal sensory nerves leads to decreases in ERSNA and natriuresis, an inhibitory renorenal reflex response.1 Not only do increases in ARNA decrease ERSNA but also reflex increases in ERSNA increase ARNA.2,4 The increased ARNA will, in turn, decrease ERSNA via activation of the renorenal reflexes, a negative-feedback mechanism, to maintain low-level ERSNA.

Changes in ERSNA modulate ARNA by the release of norepinephrine (NE), which activates $\alpha_1$-adrenoceptors (ARs) and $\alpha_2$-ARs on renal sensory nerves, leading to increases and decreases in ARNA, respectively.2 The physiologic importance of the ERSNA-induced increases in ARNA is underlined by the interaction being modulated by dietary sodium. A high-sodium diet enhances and a low-sodium diet reduces the ERSNA-induced increases in ARNA.5 The importance of the renorenal reflex−induced inhibition of ERSNA in the control of body fluid and sodium homeostasis was demonstrated in rats lacking intact afferent renal innervation. These rats are characterized by increased ERSNA, increased responsiveness of ERSNA to various sympathetic stimuli, and increased arterial pressure when fed a high-sodium diet.4

There is considerable evidence for increased ERSNA contributing to hypertension. Renal denervation reduces arterial pressure in various animal models of hypertension.1 Importantly, these studies in animals are supported by studies in humans demonstrating that bilateral renal denervation results in a long-term reduction in arterial pressure.5 The increased ERSNA most likely is related to a defect in the reflex control of ERSNA, which is diffuse and involves
the arterial and cardiac baroreceptor reflexes and the renorenal reflexes. In spontaneously hypertensive rats (SHR), there is an impairment of the responsiveness of the renal sensory nerves to various stimuli, including stretching of the renal pelvic wall and bradykinin. In normotensive rats, renal mechanosensory nerve stimulation involves bradykinin-2 receptors, leading to activation of protein kinase (PK) C, induction of cyclooxygenase-2, and increased renal pelvic synthesis of prostaglandin (PG) E2. PGE2 activates the AC/cAMP/PKA transduction pathway, with a resultant increase in substance P release and increases in ARNA. In conditions of increased ANG II activity, ANG II suppresses expression of PGE2 release. Subsequently, the renal sensory nerves in SHR involved a mechanism(s) downstream of activation of PGE2 synthesis by mechanisms currently unknown.2,8,9,10

In the current studies, increases in ERSNA were produced by thermal cutaneous stimulation by placing the rat’s tail in warm water. Because our initial studies showed reduced ARNA responses to increases in ERSNA in SHR, we then examined the mechanisms contributing to the impaired ERSNA-ARNA interaction. We first examined whether the ANG II type 1 (AT1) receptor antagonist losartan enhanced the responsiveness of the renal sensory nerves in SHR. However, losartan had no effect. Because NE-mediated activation of α2-ARs suppresses the responsiveness of the renal sensory nerves in normotensive rats2 and renal α2-AR density is increased in SHR,11 we then hypothesized that the reduced responsiveness of the renal sensory nerves to increases in ERSNA in SHR involves increased NE-mediated activation of renal pelvic α2-ARs. We tested this hypothesis by examining the effects of the α2-AR inhibitor rauwolscine alone and together with losartan on the responsiveness of the renal sensory nerves to increases in ERSNA and NE. The in vivo studies were complemented by in vitro studies to examine the effects of NE administered to an isolated, renal pelvic wall preparation to minimize possible modulation of the responsiveness of the renal sensory nerves by the hemodynamic changes produced by thermal cutaneous stimulation and to determine whether the impaired ERSNA-ARNA interaction involves a peripheral mechanism(s) at the renal sensory nerve endings.

Methods
The experimental protocols were approved by the institutional animal care and use committee. Male 10- to 12-week-old SHR, 280±5 g (range, 220 to 380 g), and Wistar-Kyoto rats (WKY), 310±20 g (range, 200 to 490 g), were anesthetized with pentobarbital sodium (0.2 mmol/kg IP, Abbott Laboratories).

In Vivo Studies
Anesthesia was maintained by pentobarbital sodium (0.04 mmol·kg⁻¹·h⁻¹) at 50 µL/min IV throughout the experiment. Arterial pressure was recorded from a catheter in the femoral artery. The left renal pelvis was perfused with vehicle or various perfusates throughout the experiment. ERSNA and ARNA were recorded from the central and peripheral portions, respectively, of the cut ends of 2 adjacent left renal nerve branches placed on bipolar silver wire electrodes. ERSNA and ARNA, integrated over 1-second intervals, were expressed as percentages of their baseline values.2,3,6–8,10 The renal sensory nerves were stimulated by reflex-mediated increases in ERSNA produced by placing the rat’s tail in 49°C water for 3 minutes.2,3 A 10-minute control and recovery period bracketed the 3-minute experimental period.

In Vitro Studies
NE was added to the incubation bath containing either the ipsilateral or the contralateral pelvies from the various rats during a 5-minute experimental period, which was bracketed by four 5-minute control periods and four 5-minute recovery periods.2,3,6–10

Experimental Protocols

**SHR and WKY In Vitro: Effects of NE on Renal Pelvic Release of PGE2 and Substance P**

In SHR (n=6) and WKY (n=8), the pelvies were incubated in buffer throughout the experiment. During the experimental period, 1 pelvis was exposed to 1250 pmol/L NE and the other to 6250 pmol/L NE in SHR; and in WKY, 1 pelvis was exposed to 50 pmol/L NE and the other to 250 pmol/L NE.

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**Figure 1.** Increasing renal pelvic pressure (RPP) or bradykinin (BK) activates PKC, which leads to increases in renal pelvic release of PGE2 (via induction of cyclooxygenase [COX]-2). PGE2 activates the AC/cAMP/PKA transduction pathway, with a resultant increase in substance P release and increases in ARNA. In conditions of increased ANG II activity, ANG II suppresses expression of PGE2 release. Subsequently, the renal sensory nerves in SHR involved a mechanism(s) downstream of activation of PGE2 synthesis by mechanisms currently unknown.2,8,9,10

**Figure 2.** ANG II activates the renal sensory nerves by stimulating α1-ARs and α2-ARs, leading to increases and decreases, respectively, in renal PGE2 synthesis by mechanisms currently unknown.2,8,9,10
SHR In Vivo: Effects of Losartan and Rauwolscine on the Release of PGE₂ and Substance P Produced by 250 and 50 pmol/L NE

In first 2 groups, 1 pelvis was incubated in buffer and the other pelvis in 0.44 mmol/L losartan (n=6) or 0.1 μmol/L rauwolscine (n=9) throughout the experiment. In the third group (n=9), 1 pelvis was incubated in rauwolscine and the other pelvis in rauwolscine+losartan throughout the experiment. In the fourth group, 1 pelvis was incubated in losartan (n=8) or rauwolscine (n=6) and the other pelvis, in rauwolscine+losartan. In the first 3 groups, both pelvises were exposed to 250 pmol/L NE and in the last group, to 50 pmol/L NE during the experimental period.

WKY In Vivo: Effects of Losartan and Rauwolscine on the Release of PGE₂ and Substance P Produced by 50 and 10 pmol/L NE

In the first group (n=6), 1 pelvis was incubated in buffer and the other pelvis in rauwolscine throughout the experiment. In the second group (n=10), 1 pelvis was incubated in rauwolscine and the other pelvis in rauwolscine+losartan. In the first group, both pelvises were exposed to 50 pmol/L NE and in the second group, to 10 pmol/L NE during the experimental period.

SHR In Vivo: Effects of Losartan and Rauwolscine on the ARNA Responses to Reflex Increases in ERSNA

The experiments were divided into 2 parts. During each part, the rat’s tail was placed in 49°C water during 2 experimental periods. Twenty minutes after the first recovery period, the renal pelvic perfusate was switched from vehicle to 0.44 mmol/L losartan (n=5), 0.1 μmol/L rauwolscine (n=6), or losartan+rauwolscine (n=6). Ten minutes later, the control, experimental, and recovery periods were repeated.

WKY In Vivo: Effects of Rauwolscine on the ARNA Responses to Reflex Increases in ERSNA

These experiments used a similar protocol, except only rauwolscine was administered into the renal pelvis (n=10) during the second part of the experiment.

Drugs and Reagents

Substance P antibody (IHC 7451) was acquired from Peninsula Laboratories (San Carlos, CA) and PGE₂, from Cayman Chemicals (Ann Arbor, MI). All other reagents/chemicals were from Sigma Aldrich (St. Louis, MO) unless otherwise stated. Washed ERSNA were dissolved in 0.1% ascorbic acid in 0.15 mol/L NaCl. All other agents were dissolved in 0.15 mol/L NaCl.

Analytic Procedures

Concentrations of substance P and PGE₂ in the incubation medium were measured by ELISA, as previously described in detail.²,³,⁹,¹⁰

Statistics

In vivo, increases in ERSNA, ARNA, mean arterial pressure (MAP), and heart rate (HR) produced by thermal cutaneous stimulation were evaluated by calculating the area under the curve of each parameter versus time, with baseline being the control period. In vitro, the increases in substance P and PGE₂ released by NE were determined by calculating the differences between the values during the experimental period and the average values of the 4 control and recovery periods. Normally distributed data (D’Agostino and Pearson omnibus normality tests) were analyzed by paired and unpaired t tests and 1-way ANOVA with repeated measures, followed by Bonferroni’s multiple comparison test. Nonnormally distributed data were analyzed by Wilcoxon signed-rank test and Friedman 1-way ANOVA with repeated measures, followed by Dunn’s multiple comparison test (GraphPad Prism 5.03, GraphPad Software, Inc, La Jolla, CA). A significance level of 5% was chosen. Data in the text and figure are expressed as mean±SE.

Effects of Reflex Increases in ERSNA and NE in SHR and WKY, In Vivo and In Vitro

To compare the responses to thermal cutaneous stimulation between SHR and WKY, the first responses in SHR, that is, in the presence of vehicle, were pooled and compared with the first responses in WKY. Thermal cutaneous stimulation increased MAP in SHR, from 143±3 to 162±3 mm Hg (peak value), and in WKY, from 112±4 to 122±4 mm Hg (both P<0.01). Although the percent increases in peak MAP, 13±1% versus 9±2%, were slightly greater (P=0.04) in SHR than in WKY, the MAP responses to thermal cutaneous stimulation were similar in SHR and WKY when calculated as the area under the curve of MAP versus time, 3090±430 and 2030±440 mm Hg·second. Likewise, there were no differences in the HR responses between SHR and WKY, 10 350±1220 and 17 240±3350 bpm·second.

The increases in ARNA produced by reflex increases in ERSNA were smaller in SHR than in WKY (P<0.01; Figure 2A). Likewise, the ERSNA responses in SHR, 6230±1021%·second, were smaller than those in WKY, 21 100±5290%·second (P<0.01). Whereas the increases in ARNA were correlated with the increases in ERSNA in WKY (r²=0.79), there was no such correlation in SHR (r²=0.14). To examine whether the reduced ARNA responses to reflex increases in ERSNA involved suppressed responsiveness of the renal sensory nerves to NE, we compared the increases in renal pelvic release of PGE₂ and substance P produced by NE added to isolated renal pelvises from SHR and WKY. In SHR, 1250 pmol/L NE failed to increase renal pelvic release of PGE₂, but the release of substance P in WKY at 250 pmol/L NE was not at 50 pmol/L, increased substance P release (Figure 2B). The increased release of substance P by 250 pmol/L NE was associated with marked increases in PGE₂ release (Table I).

Results

SHR In Vitro: Effects of Losartan and Rauwolscine on the Release of PGE₂ and Substance P Produced by 250 and 50 pmol/L NE

Our findings showing that 6250 pmol/L NE increased renal pelvic release of PGE₂ but not of substance P in SHR suggested that substance P release was suppressed by mechanisms(s) downstream of PGE₂ release. Therefore, we examined whether rauwolscine and/or losartan lowered the concentration of NE required to increase substance P release. NE at 250 pmol/L had no effect on substance P release in the presence of losartan (Figure 3A). However, in the presence of rauwolscine, 250 pmol/L NE resulted in marked increases in renal pelvic release of substance P (Figure 3B) and PGE₂ (online-only Table II available at http://hyper.ahajournals.org). In the presence of losartan+rauwolscine, the increase in substance P release produced by NE was greater than that in the presence of rauwolscine alone (P<0.05) (online-only Table III available at http://hyper.ahajournals.org). These data suggested that the combined administration of losartan...
and rauwolscine may reduce the concentration of NE required to activate the renal sensory nerves. Therefore, we examined the effects of losartan and rauwolscine on the responses to 50 pmol/L NE. NE at 50 pmol/L had no effect on renal pelvic release of PGE₂ or substance P in the presence of losartan or rauwolscine alone (Figure 4A and B and online-only Table IV available at http://hyper.ahajournals.org). However, in the presence of losartan+rauwolscine, NE increased both renal pelvic release of PGE₂ and of substance P.

**WKY In Vitro: Effects of Losartan and Rauwolscine on the Release of PGE₂ and Substance P Produced by 50 and 10 pmol/L NE**

NE at 50 pmol/L increased renal pelvic release of PGE₂ and of substance P in the presence but not in the absence of rauwolscine (Figure 4C and Table IV). However, 10 pmol/L NE had no effect on the increase in substance P release in the presence of rauwolscine alone or rauwolscine + losartan (online-only Figure I available at http://hyper.ahajournals.org).

**SHR In Vivo: Effects of Losartan and Rauwolscine on the ARNA Responses to Reflex Increases in ERSNA**

Thermal cutaneous stimulation resulted in reproducible increases in ERSNA (Figure 5, A–C), MAP, and HR (data not shown) in the 3 groups. Renal pelvic perfusion with losartan had no effect on the ARNA responses to reflex increases in ERSNA (Figure 5A). Reflex increases in ERSNA increased ARNA in the presence but not in the absence of renal pelvic perfusion with rauwolscine (Figure 5B). However, the differences between the ARNA responses in the presence of vehicle and rauwolscine did not reach statistical significance (P=0.06). In the presence of renal pelvic perfusion with rauwolscine + losartan, the ERSNA-induced ARNA responses were greater than those in the presence of vehicle (P<0.05; Figure 5C). Baseline MAP in the 3 groups, 143±5, 151±6, and 140±2, were unaffected by losartan and rauwolscine.
WKY In Vivo: Effects of Rauwolscine on the ARNA Responses To Reflex Increases in ERSNA
Thermal cutaneous stimulation resulted in reproducible increases in ERSNA (Figure 6), MAP, and HR(data not shown). Reflex increases in ERSNA resulted in similar increases in ARNA in the presence and absence of renal pelvic perfusion with rauwolscine (Figure 6). Baseline MAP, 113±4 mm Hg, was not affected by rauwolscine.

Discussion
The present results show that the interaction between ERSNA and ARNA is suppressed in SHR compared with WKY in association with reduced responsiveness of the renal sensory nerves to NE. Renal pelvic administration of losartan alone had no effect on the responsiveness of the renal sensory nerves to increases in ERSNA or NE in SHR. In SHR in the presence of rauwolscine, renal pelvic release of PGE2 and substance P from isolated renal pelvises was increased by 250 pmol/L NE, that is, the same NE concentration that increased renal pelvic release of PGE2 and substance P in WKY in the absence of rauwolscine. In the presence of losartan+rauwolscine, the threshold concentration of NE for PGE2 and substance P release was 50 pmol/L in SHR, that is, the same NE concentration that increased renal pelvic release of PGE2 and substance P in WKY in the presence of rauwolscine alone. The in vitro studies were supported by the in vivo studies that showed that ERSNA-induced increases in ARNA were of a similar magnitude as those in WKY in the presence of vehicle. Taken together, our findings suggest that the suppressed interaction between ERSNA and ARNA involves reduced responsiveness of the renal pelvic sensory nerves to NE by a mechanism(s) at the peripheral renal sensory nerve endings involving increased activation of renal α2-ARs and AT1 receptors.

Interaction Between ERSNA and ARNA
Thermal cutaneous stimulation results in a general increase in sympathetic nerve activity, as evidenced by increases in MAP, HR, and ERSNA.2,3 Our collective evidence in normal and hypertensive rats supports the view that the sympathetic neural reflex responses are a major factor in the increased blood pressure of SHR.4,5

Figure 4. In vitro effects of 50 pmol/L NE on the release of substance P from isolated renal pelvises in the presence of (A) losartan (dashed lines) or losartan+rauwolscine (dashed/dotted lines) in SHR; (B) rauwolscine (dotted lines) or losartan+rauwolscine in SHR; and (C) vehicle (solid lines) or rauwolscine in WKY. **P<0.01 vs average of control (CNT) and recovery (REC); †P<0.05, ‡P<0.01 increase in substance P release produced by NE in the presence of vehicle compared with losartan+rauwolscine (A).

Figure 5. In vivo effects of thermal cutaneous stimulation on ERSNA and ARNA in the presence of vehicle (open bar) and (A) losartan (striped bar); (B) rauwolscine (hatched bar); or (C) rauwolscine+losartan (cross-hatched bar) in SHR. *P<0.05, **P<0.01 vs baseline; †P<0.05 increase in ARNA during rauwolscine+losartan vs vehicle.
motensive rats in various dietary sodium conditions suggests that this method of increasing ERSNA allows an in vivo characterization of the peripheral mechanisms involved in the activation of the renal sensory nerves. The mechanisms involved in the ERSNA-induced increases in ARNA are similar to those activated by renal pelvic perfusion with NE administered at concentrations producing no systemic hemodynamic effects and NE added to an isolated renal pelvic wall preparation. In normotensive rats, ERSNA increases and decreases ARNA by NE-mediated activation of $\alpha_1$-AR, and $\alpha_2$-AR, respectively, in the renal pelvic area. In the current studies, the ARNA responses to reflex increases in ERSNA were reduced in SHR compared with WKY. It is unlikely that the reduced ARNA responses in SHR were related to the reduced increases in ERSNA. Whereas the ARNA responses to increases in ERSNA were correlated in WKY, there was no such correlation between the increases in ARNA and ERSNA in SHR. Furthermore, studies examining the effects of NE administered to isolated renal pelvic wall preparations from SHR and WKY supported this hypothesis. Whereas 250 pmol/L NE increased renal pelvic release of substance P in WKY, 6250 pmol/L NE had no effect on renal pelvic release of substance P in SHR. Taken together, these data suggest that the ARNA responses to thermal cutaneous stimulation in SHR are controlled/suppressed by some mechanism(s) other than the increases in ERSNA.

Mechanisms Involved in the Reduced Responsiveness of Renal Sensory Nerves in SHR: Role of Activation of Renal Pelvic $\alpha_2$-ARs

In normotensive rats, increases in ERSNA and NE activate renal sensory nerves by a PGE$_2$-dependent mechanism(s). The PGE$_2$-mediated release of substance P involves activation of the cAMP/PKA transduction pathway. In the current study, 6250 pmol/L NE failed to increase renal pelvic release of substance P, despite marked increases in renal pelvic release of PGE$_2$ in SHR, suggesting that mechanism(s) downstream of PGE$_2$ contribute to the suppressed responsiveness of the renal nerves in SHR. There is increased renal vascular responsiveness to ANG II in SHR, which has been linked to functional defects in the receptor–G-protein coupling. These findings, together with the inhibitory effects of ANG II on PGE$_2$-mediated activation of AC in SHR, suggested a role for ANG II in the impaired ERSNA-ARNA interaction in SHR. However, losartan failed to alter the ARNA responses to reflex increases in ERSNA and the release of substance P produced by 250 pmol/L NE, the threshold concentration of NE for substance P release in WKY.

Mechanisms Involved in the Reduced Responsiveness of Renal Sensory Nerves in SHR: Role of Activation of Renal Pelvic $\alpha_2$-ARs

There is extensive evidence for an altered receptor–G-protein coupling in various tissues, including renal tubular and vascular beds. Although there are conflicting reports whether the levels of $G_s$ and $G_i$ protein are altered in SHR, decreased basal and stimulated cAMP activity has been a consistent finding in both renal and cardiac tissue in SHR. $\alpha_2$-ARs are coupled to pertussis toxin–sensitive $G_{i/o}$ proteins. In the kidney, $\alpha_2A$-AR and $\alpha_2C$-AR mRNAs are expressed in the outer and inner medulla and the renal pelvic wall. Our immunohistochemical studies localized $\alpha_2A$-ARs and $\alpha_2C$-ARs on or close to the sensory nerves in the renal pelvic wall. The suppressed responsiveness of the renal sensory nerves in rats fed a low-sodium diet involves increased activation of renal pelvic $\alpha_2$-ARs. Likewise in SHR, the high concentration of NE (6250 pmol/L) required to increase PGE$_2$ release in the current studies suggests that the $\alpha_1$-AR–mediated increase in PGE$_2$ release is suppressed by a powerful NE-mediated activation of $\alpha_2$-ARs. These findings, together with the increased density of $\alpha_2$-ARs in renal tissue in SHR, suggested a role for increased activation of renal pelvic $\alpha_2$-ARs in the impaired responsiveness of the renal sensory nerves in SHR. The results of the current studies confirmed our hypothesis. Administration of rauwolscine lowered the threshold concentration of NE for PGE$_2$ and substance P release to 250 pmol/L in SHR and to 50 pmol/L in WKY. Also in the presence of rauwolscine, increases in ERSNA produced significant increases in ARNA, which were not observed in the presence of vehicle. Rauwolscine had no effect on the ARNA responses in WKY.

However, rauwolscine did not normalize the responsiveness of the renal sensory nerves in SHR. Because ANG II suppresses the PGE$_2$-mediated activation of the renal sensory nerves in SHR, we reasoned that a combination of losartan plus rauwolscine may normalize the responsiveness of the renal sensory nerves to increases in ERSNA and NE in SHR. Our initial findings showing that the release of substance P produced by 250 pmol/L NE was greater in the presence of losartan+rauwolscine than in the presence of rauwolscine.
alone supported our hypothesis. The greater increase in substance P release was not related to a greater increase in PGE$_2$, suggesting that the ANG II–induced suppression of substance P release involves a mechanism(s) downstream of PGE$_2$ synthesis. Our studies further showed that in the presence of losartan + rauwolscine, the NE threshold concentration for renal pelvic release of substance P was 50 pmol/L in SHR, that is, similar to that in WKY in the presence of rauwolscine alone. The findings that losartan only enhanced the responsiveness of the renal sensory nerves to NE in the presence of $\alpha_2$-AR blockade suggest a powerful inhibitory effect of activation of $\alpha_2$-ARs on the responsiveness of the renal sensory nerves that may surpass that of endogenous ANG II. Our studies in vivo showed that the ARNA responses to increases in ERSNA in SHR in the presence of renal pelvic perfusion with losartan + rauwolscine were similar to those in WKY in the presence of vehicle, suggesting that increased activation of both AT1 receptors and $\alpha_2$-ARs contributes to the impaired interaction between ERSNA and ARNA in SHR. It is recognized that the ERSNA-induced ARNA responses in the presence of losartan + rauwolscine were compared with those in the presence of vehicle and not with those in the presence of losartan or rauwolscine alone. However, previous pilot studies in normotensive rats showing nonreproducible increases in ARNA in response to >2 periods of thermal cutaneous stimulation made these comparisons difficult to obtain, taking into consideration the necessity to include thermal cutaneous stimulation in the presence of vehicle in each experiment. Nevertheless, our in vivo findings are supported by our in vitro studies, which allowed comparisons between the NE-induced activation of renal sensory nerves in the presence of losartan + rauwolscine with that of either agent alone.

There is little evidence for the increased renal vascular responsiveness to ANG II in SHR being related to increased AT1 receptor binding. On the other hand, there is a general agreement that the density of $\alpha_2$-ARs in renal tissue is increased in SHR. Although these data most likely represent both neural and nonneural renal tissue, an increased number of renal $\alpha_2$-ARs may contribute to the $\alpha_2$-AR–mediated impairment of the interaction between ERSNA and ARNA in SHR. The mechanisms by which rauwolscine and/or rauwolscine + losartan enhanced the responsiveness of the renal sensory nerves in SHR are currently unknown. Previous studies would suggest that $\alpha_2$-AR– and AT1 receptor–mediated coupling to pertussis toxin–sensitive G$_i$ proteins contributes to the impaired responsiveness of the renal sensory nerves to increases in ERSNA by suppressing PGE$_2$–mediated activation of AC. Although not studied in SHR, the increased PGE$_2$ release in the presence of rauwolscine may be related to cross-talk among the second-messenger systems activated by G$_i$, G$_o$, and/or G$_i$ protein–coupled receptors. Activation of G$_i$–coupled receptors has been shown to affect phospholipase C and/or PKC activity, either directly or via inhibition of the AC/cAMP transduction pathway in neuronal and nonneuronal cells.

In summary, our findings suggest that the interaction between ERSNA and ARNA is impaired in SHR due to increased activation of $\alpha_2$-ARs and AT1 receptors in renal pelvic tissue. The studies in the isolated renal pelvic wall preparation, together with our immunohistochemical studies, suggest that the suppressed ERSNA-ARNA interaction involves postsynaptic $\alpha_2$-AR, located on or close to the renal sensory nerves.

**Perspectives**

In normotensive rats, the interaction between ERSNA and ARNA serves as an important negative-feedback mechanism to maintain low ERSNA. The ERSNA-ARNA interaction is enhanced by a high- and suppressed by a low-sodium diet in the overall goal of maintaining sodium balance during various dietary sodium intakes. Modulation of renal pelvic $\alpha_2$-ARs contributes to the altered ERSNA-ARNA interaction during various dietary sodium intakes. Activation of renal pelvic $\alpha_2$-ARs is increased and decreased in low- and high-sodium diet rats, respectively. Whereas suppressed interaction between ERSNA and ARNA is a physiologically appropriate response in low-sodium dietary conditions to minimize sodium loss, an impaired ERSNA-ARNA interaction in SHR would contribute to the increased ERSNA and sodium retention, characteristics of hypertension. The impaired ERSNA-ARNA interaction in normotensive rats fed a low-sodium diet involved increased activation of renal pelvic $\alpha_2$-ARs. $\alpha_2$-AR density in renal tissue is greater in SHR than in WKY and increases further in conditions of high-sodium dietary intake in association with further increases in arterial pressure. Our current findings suggest that the impaired interaction between ERSNA and ARNA in SHR involves inappropriately increased activation of renal $\alpha_2$-ARs. Increased NE-mediated activation of $\alpha_2$-ARs on the renal sensory nerves may play an important role in the development of salt-sensitive hypertension.

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**Disclosures**

None.

**References**

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SUPPLEMENTAL MATERIAL

Impaired Interaction Between Efferent and Afferent Renal Nerve Activity in SHR Involves Increased Activation of $\alpha_2$-Adrenoceptors

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Table S1. Effects of norepinephrine on PGE$_2$ release from isolated renal pelvises derived from SHR and WKY

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<td></td>
<td>CNT</td>
<td>NE 1250 pM</td>
<td>CNT</td>
<td>NE 6250 pM</td>
</tr>
<tr>
<td></td>
<td>102±12</td>
<td>164±8</td>
<td>115±18</td>
<td>290±36†‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CNT</td>
<td>NE 50 pM</td>
<td>CNT</td>
<td>NE 250 pM</td>
</tr>
<tr>
<td></td>
<td>103±14</td>
<td>177±19*</td>
<td>94±11</td>
<td>284±43†‡</td>
</tr>
</tbody>
</table>

PGE$_2$ pg/min; CNT, average value of control and recovery; NE, norepinephrine; * P<0.05, †P<0.01 vs., CNT; ‡ P<0.05 the increase in PGE$_2$ release produced by NE at the higher concentration compared to the increase in PGE$_2$ produced by NE at the lower concentration in each group.
Table S2. Effects of 250 pM norepinephrine on PGE₂ release from isolated renal pelvises derived from SHR

<table>
<thead>
<tr>
<th>Treatment:</th>
<th>SHR, n=4</th>
<th>SHR, n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>Vehicle</td>
<td>LOS</td>
</tr>
<tr>
<td>126±13</td>
<td>109±20</td>
<td></td>
</tr>
<tr>
<td>NE 250 pM</td>
<td>142±18</td>
<td>119±22</td>
</tr>
</tbody>
</table>

PGE₂ release, pg/min; CNT, average value of control and recovery; NE, norepinephrine; LOS, losartan; RWC, rauwolscine; *P<0.05 vs., CNT
<table>
<thead>
<tr>
<th>Period:</th>
<th>Substance P, pg/min</th>
<th>PGE$_2$, pg/min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNT</td>
<td>NE 250 pM</td>
</tr>
<tr>
<td>RWC</td>
<td>5.6±0.4</td>
<td>8.9±0.8*</td>
</tr>
<tr>
<td>RWC+LOS</td>
<td>6.2±0.6</td>
<td>12.2±0.9*†</td>
</tr>
</tbody>
</table>

CNT, average value of control and recovery; NE, norepinephrine; RWC, rauwolscine; LOS, losartan; *P<0.01 vs., CNT; † P<0.01, NE-induced increase in substance P release in the presence of LOS+RWC vs., RWC, n=9
Table S4. Effects of 50 pM norepinephrine on PGE$_2$ release from isolated renal pelvises derived from SHR and WKY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SHR, n=6</th>
<th>SHR, n=8</th>
<th>WKY, n=6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RWC</td>
<td>LOS</td>
<td>Vehicle</td>
</tr>
<tr>
<td>CNT</td>
<td>110±13</td>
<td>84±5</td>
<td>94±10</td>
</tr>
<tr>
<td>NE 50 pM</td>
<td>168±21</td>
<td>110±11</td>
<td>226±31*†</td>
</tr>
</tbody>
</table>

PGE$_2$ release pg/min; CNT, average value of control and recovery; NE, norepinephrine; RWC, rauwolscine; LOS, losartan; *P<0.01 vs., CNT; † P<0.01 NE-induced increase in PGE$_2$ release in the presence of LOS+RWC vs., LOS.