Impaired substance P release from renal sensory nerves in SHR involves a pertussis toxin-sensitive mechanism

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Kopp, Ulla C., and Michael Z. Cicha. Impaired substance P release from renal sensory nerves in SHR involves a pertussis toxin-sensitive mechanism. Am J Physiol Regul Integr Comp Physiol 286: R326–R333, 2004.—Stretching the renal pelvic wall activates renal mechanosensory nerves by a PGE2-mediated release of substance P via activation of the cAMP-PKA pathway. Renal pelvic ANG II modulates the responsiveness of renal sensory nerves by suppressing the PGE2-mediated activation of adenylyl cyclase via a pertussis toxin (PTX)-sensitive mechanism. In SHR, activation of renal mechanosensory nerves is impaired. This is due to suppressed release of substance P in response to increased pelvic pressure. The present study was performed to investigate whether the PGE2-mediated release of substance P was suppressed in SHR vs. WKY and, if so, whether the impaired PGE2-mediated release of substance P was due to ANG II activating a PTX-sensitive mechanism. In an isolated renal pelvic wall preparation, PGE2, 0.14 μM, increased substance P release from 9 ± 3 to 22 ± 3 pg/min (P < 0.01) in Wistar-Kyoto rats (WKY), but had no effect in spontaneously hypertensive rats (SHR). A tenfold higher concentration of PGE2, 1.4 μM, was required to increase substance P release in SHR, from 7 ± 1 to 22 ± 3 pg/min (P < 0.01). In SHR, treating renal pelvises with losartan enhanced the release of substance P produced by subthreshold concentration of PGE2, 0.3 μM, from 16 ± 2 to 26 ± 3 pg/min (P < 0.01). Likewise, treating renal pelvises with PTX enhanced the PGE2-mediated release of substance P from 10 ± 1 to 33 ± 3 pg/min (P < 0.01) in SHR. In WKY, neither losartan nor PTX had an effect on the release of substance P produced by subthreshold concentrations of PGE2, 0.03 μM. In conclusion, the impaired responsiveness of the renal mechanosensory nerves in SHR involves endogenous ANG II suppressing the PGE2-mediated release of substance P via a PTX-sensitive mechanism.

The majority of the renal sensory nerves are located in the renal pelvic wall (26, 35). The sensory nerves are activated by increases in renal pelvic pressure of a magnitude ≥3 mmHg seen during moderate volume expansion (22). The increase in afferent renal nerve activity (ARNa) produced by the increased renal pelvic pressure leads to a reflex decrease in efferent renal sympathetic nerve activity (ERSNa) and a diuresis and natriuresis, i.e., a renorenal reflex response (28).

Among the various mechanisms activated by stretching the renal pelvic wall is stimulation of bradykinin-2 receptors, resulting in activation of protein kinase C that leads to activation of cyclooxgenase-2 and increased renal pelvic synthesis of PGE2 (25, 27). PGE2 increases the release of substance P via activation of the cAMP-protein kinase A transduction pathway (24). The PGE2-mediated release of substance P requires influx of calcium via N-type calcium channels (18). Substance P activates the afferent renal nerves by stimulating neurokinin-1 receptors in the renal pelvic area (30).

Our studies in afferent-renal-denervated rats suggest that the renorenal reflexes are an important component of the various mechanisms involved in the maintenance of water and sodium homeostasis. Arterial pressure is markedly increased in afferent renal-denervated rats in conditions of chronic high-sodium intake (21). Hence, interruption of the afferent limb of the renorenal reflexes results in the development of increased arterial pressure to facilitate natriuresis and maintenance of sodium balance during high sodium intake.

In this context, it is interesting to note that the renorenal reflexes are impaired in spontaneously hypertensive rats (SHR) (19, 29, 31), a hypertensive model characterized by increased ERSNa and sodium retention (10). The impaired increases in ARNA in response to increased renal pelvic pressure are associated with suppressed renal pelvic release of substance P in SHR (19). Because the increase in PGE2 produced by increased renal pelvic pressure was similar in SHR and Wistar-Kyoto rats (WKY), these findings suggest that the impaired renorenal reflexes are linked to mechanisms involved in the PGE2-mediated renal pelvic release of substance P.

The present studies were performed to examine the mechanisms involved in the suppressed substance P release from the renal pelvic nerves in SHR. Our initial studies were performed to examine whether the suppressed release of substance P demonstrated in vivo in response to increased renal pelvic pressure (19) could be demonstrated in response to exogenous administration of PGE2 in an isolated renal pelvic wall preparation. Because our in vitro studies showed an impaired substance P release in response to PGE2, our further studies were designed to examine the mechanisms involved in the suppressed substance P release in SHR using this in vitro model.

We focused on ANG II because our previous in vivo and in vitro studies showed that the responsiveness of the renal mechanosensory nerves was suppressed in physiological and pathophysiological models of high renin-angiotensin system activity, including low-sodium diet (22) and congestive heart failure (23). Administration of the ANG type 1 (AT1) receptor antagonist losartan into the renal pelvis restored the responsiveness of the renal mechanosensory nerves toward that in normal rats. Conversely, ANG II suppressed the responsive-

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ness of these sensory nerves in rats fed high-sodium diet. These studies showed that endogenous ANG II exerts an inhibitory effect on the PGE2-mediated release of substance P from the peripheral renal sensory nerves. Further studies showed that ANG II inhibits the PGE2-mediated activation of adenyl cyclase via a pertussis toxin (PTX)-sensitive mechanism (20), suggesting an involvement of the inhibitory guanine nucleotide binding protein (Gi) in the ANG II-mediated suppression of the PGE2-mediated substance P release.

Although there are conflicting reports on whether renal ANG II levels are increased in SHR (6, 17), there is extensive evidence for an increased renal vascular responsiveness to ANG II in SHR (e.g., Refs. 9, 32). The enhanced responsiveness in various vascular beds to ANG II has been linked to a functional defect in the receptor-G protein coupling (37, 43). Therefore, we hypothesized that the impairment of the PGE2-mediated release of substance P in SHR involved ANG II suppressing the PGE2-elicited substance P release by a PTX-sensitive mechanism. To test this hypothesis, we first examined the role of endogenous ANG II in the impaired substance P release in SHR by studying the effects of the AT1 receptor antagonist losartan on the PGE2-mediated substance P release from renal pelvices from SHR and WKY. Because these studies showed that losartan enhanced the PGE2-mediated release of substance P from renal pelvices derived from SHR but not WKY, we subsequently studied the effects of treating renal pelvices with PTX on the PGE2-mediated substance P release in SHR and WKY.

METHODS

The experimental protocols were approved by the Institutional Animal Care and Use Committee and performed according to the ‘‘Guide for the Care and Use of Laboratory Animals’’ from the National Institutes of Health.

SHR, 10–14 wk old (mean 13 ± 1 wk), and WKY, 8–14 wk old (mean 12 ± 1 wk; Taconic Farms, Germantown, NY), were anesthetized with pentobarbital sodium, 0.2 mM/kg ip (Abbott Laboratories), and both kidneys were removed. The procedures for stimulating the release of substance P from an isolated rat renal pelvic wall preparation have been previously described in detail (18, 20, 22, 24). In brief, renal pelvices dissected from the kidneys were placed in wells containing 400 μl HEPES (25 mM HEPES, 135 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, 3.3 mM D-glucose, 0.1 mM ascorbic acid, 0.1% BSA, 10 μM DL-thiophan, 1 mM Phe-Ala, 50 μM p-chloromercuricmercuryisulfonic acid, pH 7.4) maintained at 37°C. Indomethacin, 0.14 mM, was present in the incubation bath to minimize the influence of endogenous PGE2 on substance P release. Each well contained the pelvic wall from one kidney.

In all experimental preparations, except those that were pretreated for 18 h with PTX or PTX-vehicle (see below), the renal pelvic walls were allowed to equilibrate for 130 min. The incubation medium was gently aspirated every 10 min for the first 120 min and every 5 min thereafter. The medium was immediately replaced with fresh HEPES-indomethacin buffer to maintain PO2 of the medium at 160–170 mmHg. The experimental protocol consisted of four 5-min control periods, one 5-min experimental period, and four 5-min recovery periods. The incubation medium, aspirated every 5 min, was placed in siliconized vials and stored at −80°C for later analysis of substance P. Renal pelvices from SHR and WKY were run in parallel.

Effects of PGE2. 0.14 μM, on substance P release. One group of 11 SHR and one group of seven WKY were studied. During the experimental period, the ipsilateral and contralateral pelvices from each group were exposed to PGE2, 0.14 μM, dissolved in the incubation medium.

Effects of calcium in the incubation bath on substance P release. Ipsilateral pelvices from six SHR and six WKY were incubated in regular HEPES-indomethacin buffer. Contralateral pelvices from each group were incubated in calcium-free HEPES-indomethacin buffer containing 3 mM EGTA and 1 mM EDTA (18). During the experimental period, both pelvices were exposed to PGE2, 0.14 μM, dissolved in the incubation medium.

Effects of increasing concentrations of PGE2 on substance P release. These experiments were performed to determine the concentrations of PGE2 required to elicit a release of substance P of a similar magnitude in SHR and WKY. In pilot experiments, ipsilateral pelvices from three WKY rats were exposed to PGE2 at 0.14 μM and contralateral pelvices to PGE2 at 0.07 μM. In four SHR, the ipsilateral pelvis was exposed to PGE2 at 0.14 μM and the contralateral pelvis to 0.35 μM. The results showed that PGE2 at 0.07 and 0.14 μM increased substance P release in all three WKY pelvices, the increases being from 7.8 ± 3.1 to 13 ± 8 pg/min and from 9.0 ± 5.6 to 19.8 ± 7.1 pg/min, respectively. In SHR, PGE2 at 0.14 μM failed to increase substance P release, from 11.3 ± 2.9 to 10.7 ± 2.2 pg/min. PGE2 at 0.35 μM increased substance P release in three of four SHR, from 8 ± 2.4 to 12.7 ± 5.1 pg/min (n = 4, not significant [NS]), respectively. In subsequent experiments in nine WKY rats, the ipsilateral pelvis was exposed to PGE2 at the subthreshold concentration 0.03 μM and the contralateral pelvis to 0.14 μM. In nine SHR rats, the ipsilateral pelvis was exposed to PGE2 at the subthreshold concentration 0.3 μM and the contralateral pelvis to 1.4 μM.

Effects of AT1 receptor blockade on the PGE2-mediated release of substance P. The ipsilateral pelvices from 10 SHR and 10 WKY were incubated in HEPES-indomethacin buffer as described above. The contralateral renal pelvices were incubated in HEPES-indomethacin buffer until the start of the 20-min control period when losartan, 0.44 mM (22), was added to the incubation buffer. During the experimental period, ipsilateral and contralateral pelvices from the SHR rats were exposed to 0.3 μM PGE2 and ipsilateral and contralateral pelvices from the WKY rats to 0.03 μM PGE2. PGE2 was dissolved in the incubation media.

Effects of PTX on the PGE2-mediated release of substance P. In 12 SHR and 12 WKY rats, the ipsilateral renal pelvices were exposed for 18 h to circulating HEPES buffer containing 200 ng/ml PTX, 100 U/ml penicillin, and 100 μg/ml streptomycin (20). The contralateral renal pelvices were similarly treated, except PTX-vehicle (0.15 M NaCl) was present in the circulating medium instead of PTX. After the 18-h treatment with PTX or vehicle, the ipsilateral and contralateral pelvices were rinsed three times with regular HEPES buffer. The pelvices were then allowed to equilibrate in HEPES-indomethacin buffer for 70 min before the 20-min control period was started. During the experimental period, the ipsilateral and contralateral pelvices from the SHR rats were exposed to PGE2, 0.3 μM, and the ipsilateral and contralateral pelvices from WKY rats to PGE2, 0.03 μM.

Drugs. Losartan was supplied by Merck (Rahway, NJ). Substance P antibody (IHC 7451) was acquired from Peninsula Laboratories and PGE2 from Cayman Chemicals. All other agents were from Sigma Chemicals unless otherwise stated. Indomethacin was dissolved together with Na2CO3 (2:1 wt ratio) in HEPES buffer and PGE2 and losartan in incubation buffer.

Analytic procedures. Substance P in the incubation medium was measured by ELISA, as previously described in detail (18, 20, 22, 24).

Statistical analysis. Friedman two-way analysis of variance and short-cut analysis of variance were used to compare the substance P release during the experimental period with that during the control and recovery periods within each group. The Wilcoxon matched-pairs signed-rank test was used to compare the increase in substance P release from ipsilateral and contralateral renal pelvices and the Mann-Whitney U-test to compare substance P release between groups. The increase in substance P release was calculated as the difference between the value in the experimental period with the average value.
Furthermore, PGE$_2$ failed to increase substance P release in recovery. P (dashed lines). ** cubated in calcium-containing (solid lines) and Kyoto (WKY; B passive rats (SHR; H). In WKY, PGE$_2$ at the same concentration failed to increase substance P release.

** Effects of calcium in the incubation bath on substance P release.** We tested the idea that baseline release of substance P from the isolated renal pelvises in SHR is dependent on calcium. We incubated renal pelvises from WKY and SHR, from 9.0 ± 1.9 to 2.2 ± 0.4 pg/min (P < 0.02) and SHR, from 6.7 ± 0.6 to 1.8 ± 0.2 pg/min (P < 0.02). Furthermore, PGE$_2$ failed to increase substance P release in WKY in the absence of calcium in the incubation bath.

Table 1. Effects of PGE$_2$ on substance P release from an isolated renal pelvic wall preparation from WKY and SHR

<table>
<thead>
<tr>
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<th>Control</th>
<th>PGE$_2$ (0.14 μM)</th>
<th>Recovery</th>
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<tr>
<td>WKY (n = 7)</td>
<td>9.3±1.2</td>
<td>17.2±3.1*</td>
<td>9.1±1.6</td>
</tr>
<tr>
<td>SHR (n = 11)</td>
<td>11.7±1.3</td>
<td>12.7±1.9†</td>
<td>9.5±0.9</td>
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Values are mean ± SE in pg/min. Substance P release from the ipsilateral and contralateral renal pelvises was averaged. Control, average of 4 5-min control periods; Recovery, average of 4 5-min recovery periods; *P < 0.01 vs. control and recovery periods; †P < 0.02 PGE$_2$-mediated substance P release in spontaneously hypertensive rats (SHR) vs. Wistar-Kyoto rats (WKY).

** Effects of PGE$_2$, 0.14 μM, on substance P release.** We tested the notion that the impaired renal pelvic release of substance P produced by increased renal pelvic pressure in SHR (19) was related to an impairment in the release of substance P produced by PGE$_2$. In WKY, PGE$_2$, 0.14 μM, resulted in a reversible release of substance P from the isolated renal pelvises (P < 0.01, Table 1). However, in SHR, PGE$_2$ at the same concentration failed to increase substance P release.

**Effects of AT$_1$ receptor blockade on the PGE$_2$-mediated release of substance P.** To examine whether the impaired responses to activation of renal sensory nerves in SHR was due to endogenous ANG II suppressing the PGE$_2$-mediated release of substance P from the renal sensory nerves, we compared the effects of PGE$_2$ on substance P release in the presence and absence of losartan in the bath. PGE$_2$ was administered at subthreshold concentrations for substance P release, being 0.03 and 0.3 μM in WKY and SHR, respectively (Fig. 2). Whereas incubating renal pelvises from the WKY rats with losartan had no effect on the PGE$_2$-mediated release of substance P, incubating renal pelvises from SHR with losartan enhanced the responsiveness of the renal sensory nerves to PGE$_2$ (Fig. 3). In the presence of losartan, 0.3 μM PGE$_2$ elicited a reversible increase in substance P release (P < 0.01) in SHR. Losartan increased baseline renal pelvic release of substance P from 8.8 ± 1.1 to 16.0 ± 1.6 pg/min (P < 0.01) in SHR and from 9.4 ± 0.8 to 13.2 ± 1.9 pg/min (P < 0.05) in WKY.

**Effects of PTX on the PGE$_2$-mediated release of substance P.** We tested the notion that deactivation of the G$_i$ protein by PTX treatment (14) would increase the responsiveness of the renal sensory nerves to PGE$_2$ in SHR. Our results showed (Fig. 4) that treating renal pelvises with PTX enhanced the PGE$_2$-mediated release of substance P in SHR but not in WKY.

**DISCUSSION**

The results of these experiments show that there is an impairment in the PGE$_2$-mediated release of substance P from

![Fig. 1. Effects of 0.14 μM PGE$_2$ on substance P release from isolated renal pelvises from Wistar-Kyoto (WKY; A) and spontaneously hypertensive rats (SHR; B). Ipsilateral pelvises were incubated in calcium-containing (solid lines) and the contralateral pelvises in calcium-free media (dashed lines). **P < 0.01 vs. control and recovery.](http://ajpregu.physiology.org/DownloadedFrom/10.2302/0361-5239.2004.286.1.20040203-AJP-Regu-0048.R328)
renal pelvic nerves in adult SHR. Losartan added to the incubation bath containing the renal pelvic wall enhanced the PGE2-mediated release of substance P in SHR but not in WKY. Likewise, treating renal pelvic tissue with PTX enhanced the PGE2-mediated substance P release in SHR but not in WKY. These findings suggest that the impaired responsiveness of the renal sensory nerves in SHR involves endogenous ANG II suppressing the PGE2-mediated release of substance P via a PTX-sensitive mechanism.

PGE2-mediated substance P release in SHR. In SHR, increasing renal pelvic pressure or perfusing the renal pelvis with bradykinin or phorbol ester results in markedly reduced ARNA responses compared with those in WKY (19, 29), suggesting that the suppressed activation of the renal sensory nerves was linked to mechanism(s) involved in the renal PGE2 synthesis or beyond. Measurements of renal pelvic release of PGE2 and substance P showed that increasing renal pelvic pressure results in similar increases in renal pelvic release of PGE2 in SHR and WKY but markedly reduced substance P release (19). Because these studies suggested that the mechanisms governing the PGE2-mediated substance P release from the renal pelvis were altered in SHR, we turned to the isolated renal pelvic wall preparation to minimize possible confounding mechanisms that could influence the release of substance P outside the renal pelvic wall. In our initial experiments we compared the release of substance P produced by PGE2 at 0.14 μM in SHR and WKY. This concentration of PGE2 has been shown repeatedly to increase the release of substance P in normotensive Sprague-Dawley rats (18, 20, 22–24). Likewise, PGE2 resulted in a reversible release of substance P from renal pelvises derived from WKY. However, 0.14 μM PGE2 failed to increase the release of substance P in SHR. Thus these in vitro data showed an impairment in the activation of renal sensory nerves in SHR like we previously showed in vivo, demonstrating that the isolated renal pelvic wall preparation is a valid model to study the mechanisms involved in the suppressed PGE2-mediated release of substance P in SHR.

Fig. 2. Effects of 0.03 and 0.14 μM PGE2 on the release of substance P from isolated renal pelvises from WKY (A) and 0.3 and 1.4 μM PGE2 on the release of substance P from isolated renal pelvises from SHR (B). **P < 0.01 vs. control (CNT) and recovery (REC).

Fig. 3. Effects of PGE2 on the release of substance P from isolated renal pelvises from WKY (A) and SHR (B) in the absence (solid lines) and presence (dashed lines) of 0.44 mM losartan in the incubation bath. PGE2 was administered at 0.03 μM and 0.3 μM to the renal pelvises from WKY and SHR, respectively. **P < 0.01 vs. control and recovery, tP < 0.01 vs. PGE2-mediated release of substance P in the absence of losartan.
Calcium influx is essential for release of neuropeptides and transmitters from neurons (36, 44). Comparing the substance P release from renal pelvises incubated in a calcium-containing medium with that from contralateral pelvises incubated in a calcium-free medium showed that removing calcium from the incubation medium produced a 70% reduction in basal release of substance P in both WKY and SHR. These studies suggest that the release of substance P during baseline conditions reflects a physiological release of the neuropeptide from both WKY and SHR renal pelvises into the incubation bath. The low remaining release of substance P from the indomethacin-treated pelvises may reflect a low level of calcium remaining in the renal pelvic tissue. It is also possible that the low level substance P release is due to nonphysiological phenomena related to prior surgery. However, it is unlikely that this low level of substance P release would confound the interpretation of our studies in calcium-containing incubation media. First, baseline substance P release during control and recovery periods is stable, and, second, we previously provided ample evidence for the isolated renal pelvic wall preparation being a valid model to study the mechanisms involved in the activation of renal sensory nerves (20, 22–24).

Because the present studies indicated that there was a calcium-dependent release of substance P also in SHR renal pelvises, we reasoned that a higher concentration of PGE2 may increase the release of substance P in SHR. The results from the current studies confirm our hypothesis. Examining the effects of increasing concentration of PGE2 on substance P release showed that a tenfold higher concentration of PGE2, 1.4 μM, was required to increase the substance P release in SHR. The magnitude of the increase in substance P release produced by this concentration of PGE2 release was similar to that produced by PGE2, 0.14 μM, in WKY. Our previous studies in Sprague-Dawley rats fed normal sodium diet showed that the magnitude of increase in substance P release produced by 0.14 μM PGE2 was similar to that produced by 0.7 and 3.5 μM PGE2 (22), suggesting that maximum PGE2-mediated substance P release is achieved by PGE2 at 0.14 μM in normotensive rats. Because, PGE2 at 0.14 and 1.4 μM in WKY and SHR, respectively, produced an increase in substance P release of a similar magnitude, our findings would therefore suggest that the difference in sensitivity of the afferent renal nerves between WKY and SHR is related to the threshold concentrations of PGE2 for substance P release.

Role of ANG II in the impaired PGE2-mediated release of substance P in SHR. ANG II is present in renal pelvic tissue and modulated by dietary sodium (20). AT1 receptors have been localized in the renal pelvic wall (11, 13). Our previous in vivo and in vitro studies in normal rats fed low-sodium diet or rats with congestive heart failure have shown that acute renal pelvic administration of losartan restores the suppressed PGE2-mediated release of substance P toward that in normal rats (22, 23). Hence, endogenous ANG II modulates the responsiveness of the peripheral renal pelvic sensory nerves by suppressing the PGE2-mediated release of substance P from the renal pelvic sensory nerves. In SHR, there are conflicting reports on whether renal ANG II levels are increased (6, 17). However, it is well established that the renal vascular responsiveness to ANG II is enhanced in SHR (9, 32). The number of AT1 receptors in glomeruli, cortex, and proximal tubular cells are similar in adult SHR and WKY (2, 9, 40). Interestingly, increased ANG II binding has been reported in the renal medulla in adult SHR vs. WKY (2, 40). Chronic treatment with converting enzyme inhibitors prevents the impairment of the renorenal reflexes in SHR (31). It is also well known that blockers of the renin-angiotensin system restore the impaired baroreceptor reflex control of ERSNA in SHR in association with lowering arterial pressure (e.g., Refs. 5, 33). Thus the collective evidence in normotensive and hypertensive rats would suggest that the impairment of the renorenal reflexes in SHR might be related to ANG II exerting an enhanced inhibitory effect on the PGE2-mediated release of substance P. We tested this idea by comparing the effects of losartan on the PGE2-mediated release of substance P in SHR and WKY. Whereas losartan had no effect on the release of substance P produced by 0.03 μM PGE2 in WKY, it enhanced the release of substance P in SHR produced by 0.3 μM PGE2. It is unlikely that losartan would have enhanced the release of substance P produced by higher concentrations of PGE2 in WKY. Our previous studies showed that losartan has no effect.
on the release of substance P produced by PGE2 at 0.14 or 0.7 μM in Sprague-Dawley rats using a similar renal pelvic wall preparation (22). These studies suggest that the impaired PGE2-mediated release of substance P in SHR is mediated, at least in part, by ANG II exerting a suppressing effect on the release of substance P from the renal sensory nerve fibers in the renal pelvic wall.

Losartan produced a marked increase in baseline substance P release in SHR but only a minor increase in WKY. Our previous studies (22) showed that losartan produced an increase in baseline substance P release by a magnitude that was related to the level of endogenous ANG II. Taken together, these studies suggest that endogenous ANG II exerts an inhibitory effect on baseline substance P that is independent of PGE2 synthesis, all pelvices being indomethacin treated. The mechanisms involved in the ANG II-mediated inhibition of baseline substance P release during PG synthesis inhibition are currently not known. It is possible that the effects of losartan on baseline substance P release are related to an inhibition of ANG II-induced reduction of calcium channel activity (3, 38).

Alternatively, baseline substance P release may be modulated by reactive oxygen species (ROS) in conditions of high endogenous ANG II activity. ANG II stimulates the production of ROS in various tissues, including central nervous system and renal tissue (e.g., Refs. 7, 46), and ROS has been shown to suppress baroreceptor activity (8).

Role of the G, protein in the ANG II-mediated suppression of 
substance P in SHR. In physiological conditions of high endogenous renal ANG II levels, the PGE2-mediated release of substance P is suppressed by ANG II activating renal pelvic AT1 receptors coupled to the G, protein in Sprague-Dawley rats (20). Albeit most studies report similar AT1 receptor affinity in SHR (9, 45), there is extensive evidence for an altered receptor-G protein coupling in various tissues, including renal tubular and vascular beds (12, 37, 43). Although there are conflicting reports whether the levels of Gs and Gi protein are altered in SHR (4, 34, 43), decreased basal and stimulated cAMP activity has been a consistent finding in both renal and cardiac tissue in SHR (34, 43). These studies led us to hypothesize that the impaired PGE2-mediated release of substance P in SHR may be related to increased activation of the G, protein signaling pathway by ANG II. We tested this notion by comparing the PGE2-mediated substance P release from PTX-treated and vehicle-treated renal pelvices from SHR and WKY. Our previous studies in Sprague-Dawley rats showed that PTX pretreatment of renal pelvices prevents the inhibitory effects of exogenous ANG II on the PGE2-mediated release of substance P (20). PGE2 was administered at subthreshold concentrations for substance P release, 0.03 and 0.3 μM in WKY and SHR, respectively. PTX increased the responsiveness of the renal sensory nerves in SHR but had no effect in WKY. It is unlikely that the lack of an effect of PTX on the PGE2-mediated substance P release from WKY pelvices was related to the lower concentration of PGE2 administered because our previous studies showed that PTX had no effect on the release of substance P produced by PGE2, 0.3 μM, in Sprague-Dawley rats fed high-sodium diet, i.e., the same concentration administered to the SHR pelvices in the current study. The preventive effect of PTX of the angiotensin-mediated inhibition of the release of substance P produced by PGE2 in normotensive rats fed high-sodium diet (20) plus the lack of effect of PTX on the PGE2-mediated release of substance P in normotensive Sprague-Dawley (20) and WKY rats with low endogenous ANG II suggest that the effects produced by PTX in SHR are due to inhibition of increased ANG II activity on substance P release. Thus our findings suggest that deactivation of the G, protein enhances the responsiveness of the renal sensory nerves to PGE2 in SHR.

These findings together with our previous studies (20) would suggest that ANG II exerts its inhibitory effect by suppressing the PGE2-mediated activation of adenylyl cyclase. Whether the interaction between PGE2 and ANG II governing the release of substance P takes place within the sensory nerves per se or in the uroepithelium and/or pelvic muscle layer surrounding the renal sensory nerves cannot be deduced from these studies. However, the localization of AT1 receptors in the renal pelvic wall (11, 13) together with the demonstration of AT1 receptors on neurons in the central nervous system involved in the control of the cardiovascular system (1) may suggest the presence of AT1 receptors on the renal sensory nerves. On the basis of evidence from studies in dorsal root ganglia showing that sensory nerves can be a source of PGE2 (42) plus the demonstration of receptor-G, protein complexes in sensory nerves (15, 16), we speculate that the inhibitory effects on PGE2-mediated substance P release exerted by ANG II are due to ANG II activating AT1 receptors coupled to G, proteins on the renal sensory nerves.

Our current studies would suggest that there is an interaction between PGE2 and ANG II in the activation of adenylyl cyclase involved in the activation of renal sensory nerves after increased renal pelvic pressure (Fig. 5). The resultant activation of the renal sensory nerves would depend on the level of

\[ \text{Renal Pelvic Pressure} \]

\[ \text{PTX} \rightarrow \text{G, Protein} \rightarrow \text{Cyclase} \rightarrow \text{cAMP} \]

\[ \text{Substance P} \quad \text{PKC} \quad \text{PKA} \]

\[ \text{Afferent Renal Nerve Activity} \]

\[ \text{Efferent Renal Sympathetic Nerve Activity} \]

\[ \text{Urinary Sodium Excretion} \]

Fig. 5. In the sequence of events elicited after an increase in renal pelvic pressure, PGE2 exerts a stimulatory and ANG II an inhibitory effect on the activation of adenylyl cyclase. Interaction between PGE2 and ANG II determines the level of activation of the renal mechanosensory nerves. In conditions of low endogenous ANG II, there is an enhanced activation of the renal mechanosensory nerves resulting in decreased efferent renal sympathetic nerve activity (ERSNA) and a natriuresis. Conversely, in conditions of high endogenous ANG II, the activation of renal mechanosensory nerves is impaired resulting in increased ERSNA and sodium retention (20, 22, 24, 27).
PGE2 and ANG II activity. In conditions of high ANG II activity, including low-sodium diet, congestive heart failure, and spontaneous hypertension, the ANG II-inhibitory influence on adenyl cyclase would impair the activation of afferent renal nerves, which would lead to increased ERSNA and sodium retention. In this context, it is interesting to note that the threshold for activation of the renal mechanosensory nerves is <3 mmHg in rats fed high-sodium diet, whereas it is 7.5 mmHg in low-sodium diet-fed rats and congestive heart failure rats and >15 mmHg in SHR (19, 22, 23).

In summary, the present study shows that renal pelvic release of substance P produced by PGE2 is suppressed in SHR. Compared with WKY, a tenfold higher concentration of PGE2 and ANG II activity. In conditions of high ANG II concentration in low-sodium diet-fed rats and congestive heart failure rats and spontaneous hypertension, the ANG II-inhibitory in effects were produced by treating the renal pelvises with PTX and spontaneous hypertension, the ANG II-inhibitory in effects were produced by treating the renal pelvises with PTX for 18 h. Taken together, these data suggest that ANG II suppresses the PGE2-mediated release of substance P via a PTX-sensitive mechanism. Furthermore, our current findings may support the notion that the prevention of the development of hypertension in SHR by a converting enzyme inhibitor (31) was, at least in part, due to blocking the inhibitory effects of ANG II on the renal reflexes.

GRANTS
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REFERENCES


41. Tate MW and Clelland RC. Nonparametric and Shortcut Statistics in the Social, Biological Sciences. Danville, IL: Interstate; 1957.


