Renal Substance P–Containing Neurons and Substance P Receptors Impaired in Hypertension

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Abstract—In normotensive rats, increased renal pelvic pressure stimulates the release of prostaglandin E and substance P, which in turn leads to an increase in afferent renal nerve activity (ARNA) and a contralateral natriuresis, a contralateral inhibitory renorenal reflex. In spontaneously hypertensive rats (SHR), increasing renal pelvic pressure failed to increase afferent renal nerve activity. The inhibitory nature of renorenal reflexes indicates that impaired renorenal reflexes could contribute to increased sodium retention in SHR. Phorbol esters, known to activate protein kinase C, increase afferent renal nerve activity in Wistar-Kyoto rats (WKY) but not in SHR. We examined the mechanisms involved in the impaired responses to renal sensory receptor activation in SHR. The phorbol ester 4β-phorbol 12,13-dibutyrate increased renal pelvic protein kinase C activity similarly in SHR and WKY. Increasing renal pelvic pressure increased afferent renal nerve activity in WKY (27±2%) but not in SHR. Renal pelvic release of prostaglandin E increased similarly in WKY and SHR, from 0.8±0.1 to 2.0±0.4 ng/min and 0.7±0.1 to 1.4±0.2 ng/min. Renal pelvic release of substance P was greater (P<.01) in WKY, from 16.3±3.8 to 41.8±7.4 pg/min, than in SHR, from 9.9±1.7 to 17.0±3.2 pg/min. In WKY, renal pelvic administration of substance P at 0.8, 4, and 20 µg/mL increased ARNA 382±69, 750±233, and 783±124% per second (area under the curve of afferent renal nerve activity versus time). In SHR, substance P at 0.8 to 20 µg/mL failed to increase ARNA. These findings demonstrate that the impaired afferent renal nerve activity response to increased renal pelvic pressure is related to decreased release of substance P and/or impaired activation of substance P receptors. (Hypertension. 1998;31:815-822.)

Key Words: afferent renal nerve activity ■ receptors, sensory ■ prostaglandins ■ protein kinase C ■ substance P ■ rats, inbred SHR

Obstruction to urine flow increases renal pelvic pressure and activates renal mechanoreceptors, resulting in an increase in ipsilateral ARNA.1–4 The increase in ARNA produces a fall in contralateral efferent renal sympathetic nerve activity (ER-SNA) and a contralateral diuresis and natriuresis, known as the contralateral inhibitory renorenal reflex.2

Accumulating evidence indicates that the renal nerves contribute to the pathogenesis of hypertension in SHR.3 Peripheral sympathetic nerve activity and, in particular, ER-SNA is enhanced in SHR. The nature of the renorenal reflex, that is, a diuresis and natriuresis in association with decreased ER-SNA, would suggest that an attenuation of this reflex would result in increased ERSNA, leading to water and sodium retention, factors known to contribute to the hypertensive process.7 Our previous studies in SHR demonstrated that increasing renal pelvic pressure failed to increase ARNA and thus failed to elicit a contralateral renorenal reflex in these rats.8 The lack of an increase in ARNA in response to increased renal pelvic pressure suggested that the impairment of the renorenal reflexes in SHR is due at least in part to a peripheral defect at the level of the sensory receptors in the renal pelvis. This impaired responsiveness of renal sensory receptors was not unique to increased renal pelvic pressure because the renorenal reflex in response to renal pelvic perfusion with bradykinin10 was also impaired in SHR.11

Bradykinin is known to activate sensory receptors in various tissues by stimulating phosphoinositidase C, leading to increased intracellular calcium and activation of PKC.12,13 Activation of PKC has been shown to stimulate the phospholipase A2–dependent formation of prostaglandins.12,13 Recent in vitro studies in dorsal root ganglionic neurons showed that PKC activation also increases the release of substance P.14 In the renal pelvis, activation of PKC increased ARNA and blockade of PKC activity reduced the ARNA response to bradykinin in normotensive rats.15 Taken together, these studies indicate that the cellular mechanisms activated by renal sensory receptor activation involve the activation of PKC, leading to the release of PGE and substance P.

Signaling by the phosphoinositidase C–PKC pathway has been shown to be altered in SHR.15 In our previous studies we demonstrated that treatment with the PKC activator PDBu failed to increase ARNA in SHR.11 These data suggested that the impaired ARNA response to renal sensory receptor activation in SHR was localized to a defect in PKC or to signaling...
mechanisms beyond PKC. The present study was therefore undertaken to further explore the mechanisms underlying the decreased responsiveness of renal sensory receptors in SHR. We examined whether the PDBu-mediated activation of PKC in the renal pelvic wall and the renal pelvic release of PGE and substance P produced by increased renal pelvic pressure were altered in SHR compared with the normotensive WKY. Due to the crucial role of renal pelvic substance P receptors in the activation of renal sensory neurons by various stimuli, we also compared the responsiveness of the renal pelvic sensory receptors with substance P in WKY and SHR.

Methods

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

In Vitro Study

Group 1: Activation of PKC in the Isolated Renal Pelvis

In 10 male 10- to 13-week-old SHR and WKY (Taconic Farms, Germantown, NY) anesthetized with pentobarbital sodium (50 mg/kg IP), the left and right kidneys were removed and placed in ice-cold phosphate-buffered saline (PBS). The renal pelvic wall was dissected and placed in HEPES buffer (HEPES 25 mmol/L, NaCl 135 mmol/L, KCl 3.5 mmol/L, CaCl₂ 2.5 mmol/L, MgCl₂ 1 mmol/L, d-glucose 3.3 mmol/L, and 0.1 mmol/L acetic acid, pH 7.45) containing either the PKC activator PDBu at 1 µmol/L or vehicle (0.1% DMSO). The ipsilateral and contralateral pelvices from each of two different rats were placed in separate vials and incubated with either PDBu or DMSO, respectively, for 10 minutes at 37°C.

Measurement of PKC Activity

Activation of PKC was assessed by measuring its translocation from the cytosol to the plasma membrane as previously described. After incubation with PDBu and DMSO, respectively, the renal pelvices were rinsed in ice-cold PBS, then placed into ice-cold homogenizing buffer (Tris 20 mmol/L, EGTA 2 mmol/L, EDTA 2 mmol/L, sucrose 250 mmol/L, phenylmethylsulfonyl fluoride 1 mmol/L, and leupeptin 60 µmol/L, pH 7.5) and homogenized for 20 seconds at 15 000 rpm, with a Brinkmann polytron (Brinkmann Instrument Corp). The resulting homogenate was centrifuged at 1000 g for 10 minutes at 5°C to pellet cell debris and nuclei. The supernatant was removed and again centrifuged at 100 000 g for 30 minutes at 4°C to obtain the cytosolic fraction (supernatant). The particulate (membrane) fraction was re suspended in the homogenizing buffer containing 0.1% Triton X-100 and PKC extracted with repeated vortexing. PKC activity in the membrane and particulate fraction was assayed by measuring 32P incorporation into the substrate histone IIIs. Aliquots (5 µL) of the eluted enzyme were incubated at 30°C for 1.5 minutes in a reaction mixture containing Tris 50 mmol/L (pH 7.5), histone IIIs 50 µg, MgCl₂ 12.5 mmol/L, CaCl₂ 0.24 mmol/L, phosphatidylylserine 77 µmol/L, phorbol 12-myristate 13-acetate (PMA) 20 nmol/L, and ATP 2.5 nmol containing ~10⁶ cpm of [γ⁻³²P]ATP (final volume, of 90 µL). The reaction was terminated with 10 µL 75 mmol/L phosphoric acid, and 75 µL from each sample was spotted onto Whatman P81 phosphocellulose paper. The paper was washed three times in 75 mmol/L phosphoric acid and once in absolute ethanol. Incorporation into histone IIIs was determined by liquid scintillation counting. PKC activity, normalized for protein content and time of incubation, was calculated as the stimulated activity determined in the presence of calcium, phosphatidylylserine, and PMA minus the basal activity determined in the absence of activators. Protein content was determined with the Bio-Rad DC Protein Assay (Bio-Rad Laboratories).

In Vivo Study

The study was performed on male 10- to 14-week-old SHR (mean age, 12±0.1 weeks) weighing 275 to 355 g (mean weight, 303±2 g) and 10- to 13-week-old WKY (mean age, 11±0.2 weeks) weighing 279 to 452 g (mean weight, 361±9 g). Anesthesia was induced with pentobarbital sodium, 0.2 mmol/kg IP (Abbott Laboratories), and maintained with an intravenous infusion of pentobarbital sodium, 0.04 mmol/kg per hour IV in isotonic saline at 50 mL/min. Catheters were placed in the femoral artery for continuous arterial pressure recordings and in the femoral vein for pentobarbital sodium infusion. Heart rate was recorded with a linear cardiotachometer triggered by the arterial pressure waveform.

All recordings were made on a Grass 7D polygraph that was connected to an IBM PS/2 by a Data Translation A/D board (model DT2801) for on-line data acquisition.

A left flank incision was performed and a PE-10 catheter was inserted into the ureter for collection of urine.

Renal Pelvic Administration of Experimental Agents

A PE-60 catheter was placed in the left renal pelvis through the ureter. To administer various agents into the left renal pelvis, a PE-10 catheter was inserted into the PE-60 catheter and advanced into the renal pelvis so that its tip extended 1 to 2 mm beyond the tip of the PE-60 catheter. The renal pelvis was perfused at 20 µL/min. In group 3, the renal pelvic effluent was drained through the PE-60 catheter.

Collection of Renal Pelvic Effluent for PGE and Substance P Determination

In group 2, a pulled PE-50 catheter was inserted through the renal parenchyma into the renal pelvis to collect renal pelvic effluent. The open end of the PE-60 ureteral catheter was clamped to allow drainage of all effluent through the pulled PE-50 catheter inserted through the renal parenchyma.

Increased Renal Pelvic Pressure

Renal pelvic pressure was increased by raising the PE-60 catheter, inserted into the left ureter, above the level of the kidney while clamping the catheter inserted through the renal parenchyma. The PE-60 catheter was filled with 0.15 mol/L NaCl. Renal pelvic pressure was recorded with a P23DB Statham transducer connected to the ureteral catheter by a T-tube connector.

Recording of Afferent Renal Nerve Activity

One renal nerve branch was isolated at the angle between the aorta and the left renal artery and placed on a bipolar silver wire electrode for recordings of multifiber renal nerve activity. The signals were led by a high impedance probe (Grass HIP511) to a bandpass amplifier (Grass P511) with a high-frequency cutoff at 3000 Hz and a low-frequency cutoff at 30 Hz; they were amplified 20 000 times. The output of the bandpass amplifier was fed to an oscilloscope (Tektronix 5113) and to a resetting voltage integrator (Grass 7P10). Renal nerve activity was integrated over 1-second intervals, the unit of measure being microvolts per second per second. Assessment of renal nerve activity was done by its pulse-synchronous rhythmicity. After identification and verification of renal nerve activity, the renal nerve was sectioned and the distal part placed on the electrode for recording ARNA. The electrode was fixed to the renal nerve with silicone cement (Wacker Sil-Gel 604, Wacker-Chemie). Postmortem renal
nerve activity, which was assessed by crushing the decentralized renal nerve bundle peripheral to the recording electrode, was subtracted from all values of renal nerve activity. ARNA was expressed in percentage of its baseline value during the control period.1,5,8-11

Experimental Protocols

Approximately 1.5 hours elapsed after the end of surgery and the start of the experiment to allow the rat to stabilize as evidenced by 30 minutes of steady-state urine collections and ARNA recordings. The study was divided into two groups (groups 2 and 3). In group 2, the effects of increasing renal pelvic pressure on renal pelvic release of substance P and PGE were compared in SHR and WKY. In group 3, the effects of renal pelvic administration of increasing concentrations of substance P on ARNA were compared in SHR and WKY.

**Group 2: In Vivo Substance P and PGE Release**

In 18 SHR and 14 WKY, a 3-minute experimental period during which renal pelvic pressure was increased was bracketed by a 10-minute control and recovery period. The left renal pelvis was perfused with 0.15 mol/L NaCl containing 10 μmol/L of the endopeptidase inhibitor thiorphan to minimize the catabolism of substance P.29 Renal pelvic effluent from the left perfused kidney was collected on ice throughout the experiment and stored at −80°C for later analysis of substance P and PGE.5,10 The effects of increased renal pelvic pressure on substance P and PGE release into the renal pelvis were determined from a 1-minute collection immediately after the PE-60 ureteral catheter was lowered to the kidney level. At the end of the experiment, 2.5 μg/mL capsaicin was administered into the renal pelvis to examine the viability of the renal pelvic sensory nerves in SHR and WKY.

**Group 3: In Vivo Substance P Dose-Response Curve**

In 17 SHR and 11 WKY rats, substance P was administered into the renal pelvis at 0.16, 0.8, 4.0, 20, and 100 μg/mL in a volume of 50 μL during five experimental periods separated by 35-minute intervals. Each 5-minute experimental period was bracketed by a 5-minute control and recovery period. The renal pelvis was perfused with 0.15 mol/L NaCl (vehicle) when substance P was not administered. In addition, renal pelvic pressure was increased for 5 minutes at the beginning and end of the experiment. At the very end of the experiment, that is, after the increase of renal pelvic pressure, 2.5 μg/mL capsaicin was administered into the renal pelvis to test the viability of the renal pelvic sensory nerves in SHR and WKY.

**Drugs**

PDBu, thiorphan, substance P, and all agents used in the PKC assay except γ-32P ATP were from Sigma Chemical Co. γ-32P ATP was from Dupont-New England Nuclear. PDBu was dissolved in DMSO and further diluted with 0.15 mol/L NaCl to a final DMSO concentration of 0.1%. Thiorphan was dissolved in 100% ethanol and further diluted with 0.15 mol/L NaCl to a final ethanol concentration of 0.1%. Substance P was dissolved in 0.15 mol/L NaCl.

**Analytical Procedures**

Contralateral (right) urinary sodium concentrations were determined with a flame photometer. Right urinary sodium excretion was expressed per gram of kidney weight.

**PGE Radioimmunoassay**

PGE concentration was determined by specific RIA previously established and validated.1,5,10,21 Urine samples were assayed at three dilutions. The PGE antibody used (Iowa RAB 66) cross-reacted 100% with PGE, and PGE2, but showed <2% cross-reactivity with other arachidonic acid metabolites. The intra-assay and interassay coefficients of variation averaged 8%.

**Substance P Radioimmunoassay**

RIA for substance P was performed as previously described.1,5 The purity of substance P was assessed in three thin-layer chromatography systems, electrophoresis, amino acid analysis and two separate high-performance liquid chromatography solvent systems (Peninsula Labs). The substance P antibody (RIN 7451, Peninsula Labs) demonstrated 100% cross-reactivity with fragments 2 to 11, 3 to 11, 4 to 11, 5 to 11, <5% with 6 to 11, and <1% with fragment 7 to 11, neuropeptide K, neurokinins B and A, endothelin-1, somatostatin, and VIP. The renal pelvic effluent was assayed at three dilutions. After addition of antibody and subsequent incubation overnight at 4°C, 125I-labeled substance P (Dupont-New England Nuclear) was added to the experimental samples and pure standards. The antibody bound was separated from free substance P by adding goat anti-rabbit gamma globulin (Peninsula Labs) and diluted normal rabbit serum. The intra-assay and interassay coefficients of variation averaged 10%.

**Statistical Analysis**

**In Vitro Studies**

The effects of PDBu on the translocation of PKC from the cytosol to the membrane fraction of the renal pelvises were analyzed by comparing the fraction of total PKC activity that was membrane bound in the PDBu-treated and vehicle-treated (DMSO) pelvises, that is, (membrane PKC activity×100)/membrane PKC activity+cytosolic PKC activity.

**In Vivo Studies**

Systemic hemodynamics and renal excretion were measured and averaged over each period. In group 2, ARNA was calculated over each period and the effects of increased renal pelvic pressure on ARNA were calculated by comparing the experimental value with the average value of the bracketing control and recovery periods. In group 3, the ARNA response to substance P increased in duration with increasing concentration. Therefore, the ARNA response to substance P was calculated as the area under the curve of time vs ARNA (AUC), where ARNA was expressed as a percentage of its baseline value during the 10-minute control period preceding each experimental period. Release of substance P and PGE into the renal pelvic effluent was calculated as concentration times volume divided by duration of the collection period. The amount of substance P and PGE in the renal pelvic effluent collected during the first minute after the experimental period was corrected for baseline release and divided by the duration of the experimental period (3 minutes).

Friedman two-way ANOVA with multiple comparisons between groups was used to test which of the various concentrations of substance P increased ARNA above baseline. Fisher’s exact probability test was used to examine whether the ARNA responses to substance P at various concentrations differed between SHR and WKY. The Mann-Whitney U test and Wilcoxon matched-pairs signed-rank test were applied to test the significance between two unrelated and two related samples, respectively.22 A significance level of 5% was chosen. Data in text, tables, and figures are expressed as mean±SE.

**Results**

**In Vitro Study**

**Group 1: Activation of PKC in the Isolated Renal Pelvis**

The results are shown in Fig 1 and the Table. The renal pelvis from vehicle-treated SHR and WKY had similar PKC activity in both the cytosol and membrane fractions (Table). Compared with vehicle treated pelvises, treatment with PDBu produced reciprocal decrease in cytosolic and increase in membrane bound PKC activity, consistent with translocation and activation of PKC. Activation of PKC, as measured by the increase in the percent of total PKC that was membrane bound, was similar in WKY and SHR, 28±5% and 24±2%, respectively (Fig 1).
In Vivo Study

Increasing renal pelvic pressure failed to increase ARNA in 30 of the 35 SHR studied. Since the aim of the study was to examine the mechanisms involved in the decreased responsiveness of renal pelvic mechanosensitive neurons in SHR, the five SHR that responded with an increase in ARNA to increased renal pelvic pressure are discussed separately.

Group 2: In Vivo Substance P and PGE Release

The results are shown in Figs 2 and 3. In WKY, basal ARNA was 1059.43 μV·s·1 s⁻¹. Increasing renal pelvic pressure 16±0.3 mm Hg increased ARNA 27±2% (P<0.01, Fig 2) and contralateral urinary sodium excretion 48±15% (P<0.01). The increase in ARNA was associated with a reversible increase in ipsilateral renal pelvic release of substance P of 25.4±8.2 pg/min (P<0.01) and PGE of 1.2±0.4 ng/min (Fig 2). Mean arterial pressure, 107±3 mm Hg, and heart rate, 346±14 beats per minute, remained unaltered throughout the experiment.

In SHR, basal ARNA was 1056±51 μV·s·1 s⁻¹. In 16 of 18 SHR, increasing renal pelvic pressure 15±2 mm Hg failed to increase ARNA, 0±1% (Fig 3), or contralateral urinary sodium excretion, 11±6% (NS). Increasing renal pelvic pressure resulted in a reversible increase in ipsilateral renal pelvic release of substance P of 7.1±3.3 pg/min (P<0.01) and PGE of 0.7±0.2 ng/min (P<0.01) (Fig 3). The increase in renal pelvic release of substance P produced by increased renal pelvic pressure in SHR was less than that observed in WKY (P<0.01). However, the increased release of PGE was similar in SHR and WKY. Mean arterial pressure, 155±5 mm Hg, and heart rate, 362±13 beats per minute, remained unaltered throughout the experiment.

Group 3: In Vivo Substance P Dose-Response Curve

The results are shown in Figs 4, 5, and 6. In WKY, basal ARNA was 1203±64 μV·s·1 s⁻¹. Increasing renal pelvic pressure 20±1 and 19±2 mm Hg in the beginning and end of the experiment produced similar increases in ARNA, 25±2% and 19±3% (both P<0.01), and contralateral urinary sodium excretion, 29±7% (P<0.01) and 15±4% (P<0.02). Renal pelvic administration of substance P increased ARNA in a concentration-dependent fashion (Figs 4 and 5). The duration of the response to substance P at 0.16, 0.8, 4, and 100 μg/mL was 5±3, 23±5, 40±9, 47±7, and 78±11 seconds, respectively. Mean arterial pressure, 107±3 mm Hg and heart rate, 349±19 beats per minute, were not affected by substance P.

In SHR, basal ARNA was 1224±88 μV·s·1 s⁻¹. In 14 of 17 SHR, increasing renal pelvic pressure 21±1 and 21±1 mm Hg in the beginning and end of the experiment failed to increase ARNA (3±1% and 1±1%, respectively) or contralateral urinary sodium excretion (-15±9% and 1±4%, respectively). Renal pelvic administration of substance P at 0.8, 4, and 20 μg/mL increased ARNA in only 3, 4, and 6, of 14 SHR, respectively, which was significantly different (P<0.01) from WKY, in which substance P at 0.8, 4, and 20 μg/mL increased ARNA in all 11 WKY (Figs 4 and 6). In only 3 of 14 SHR did substance P at 100 μg/mL result in an increase in ARNA that was within the range of that in WKY (Fig 5). The number of SHR that responded to substance P at 100 μg/mL was also less than in WKY (P<0.05, Fig 6). Mean arterial pressure, 152±4 mm Hg and heart rate, 324±9 beats per minute, were not significantly affected by substance P.

### Table: Protein Kinase C Activity in the Cytosol and Membrane Fractions of Isolated Renal Pelvic Wall Preparations in WKY and SHR

<table>
<thead>
<tr>
<th>PKC Activity, nmol [32P]·mg⁻¹·min⁻¹</th>
<th>DMSO</th>
<th>PDBu, 1 μmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytosol</td>
<td>Membrane</td>
</tr>
<tr>
<td>WKY (n=10)</td>
<td>0.72±0.08</td>
<td>0.61±0.06</td>
</tr>
<tr>
<td>SHR (n=10)</td>
<td>0.74±0.09</td>
<td>0.66±0.07</td>
</tr>
</tbody>
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*P<0.05, †P<0.01, PDBu- vs DMSO-treated pelvices.
The impaired ARNA responses to increased renal pelvic pressure and renal pelvic administration of substance P in SHR were not due to a generalized desensitization of the renal pelvic nerves since renal pelvic administration of capsaicin increased ARNA in all SHR, the ARNA responses being in the range of those in WKY (Fig 7).

In 5 of 35 SHR, increasing renal pelvic pressure resulted in an increase in ARNA that was within the range of that in WKY (25±4%). In these SHR, increasing renal pelvic pressure resulted in an increase in renal pelvic release of substance P and PGE (10±5 pg/min and 1.4±1.1 ng/min, respectively, n=2), and renal pelvic administration of substance P at 0.16, 0.8, 4, 20, and 100 µg/mL increased ARNA 0, 169±85, 865±331, 2126±1012, and 1007±388% ± seconds (n=3). Pooling the data from the SHR that responded to increased renal pelvic pressure with an increase in ARNA with those that did not respond to increased renal pelvic pressure did not alter the overall conclusion derived from the study. When the data from all SHR were pooled, increasing renal pelvic pressure failed to increase ARNA (2±2%, P<.01, 18 SHR versus 14 WKY). The renal pelvic release of PGE increased 0.8±0.2 ng/min (NS versus WKY) and substance P 7.4±2.9 pg/min (P<.01 versus WKY). Likewise, the number of SHR that responded to substance P was significantly less than the number of WKY (P<.01, 17 SHR versus 11 WKY for substance P 0.8 to 20 µg/mL and P<.05 versus WKY for substance P 100 µg/mL).

**Discussion**

The results of the present study show that PDBu increased PKC activity in the membrane fraction of an isolated renal pelvic wall preparation to a similar extent in SHR and WKY. Increasing renal pelvic pressure increased ipsilateral ARNA, ipsilateral renal pelvic release of substance P and PGE, and contralateral urinary sodium excretion in WKY. However, increasing renal pelvic pressure to the same extent in SHR failed to increase ipsilateral ARNA and contralateral urinary sodium excretion in the majority of SHR. The impaired ARNA response to increased renal pelvic pressure in SHR was...
associated with decreased release of substance P into the renal pelvic effluent. The renal pelvic release of PGE was not different from that in WKY. Furthermore, renal pelvic administration of substance P failed to increase ARNA in the majority of SHR at concentrations that produced marked increases in WKY. Our previous studies in normotensive rats showed that substance P elicits a similar renorenal reflex response as increased renal pelvic pressure.4 Furthermore, treatment with a substance P receptor antagonist or capsaicin, which depletes sensory neurons of substance P, blocked the ARNA response to increased renal pelvic pressure.4,16 Taken together, the results of our previous and current studies suggest that the impaired responsiveness of renal pelvic sensory receptors in SHR is not related to a defect in PKC activation but rather to decreased renal pelvic release of substance P and/or impaired activation of renal pelvic substance P receptors.

Previous studies have suggested that the phosphoinositide C–PKC signaling pathway may be altered in SHR.17 PKC is widely distributed in various tissues and organs. The high concentration of PKC in the nervous system compared with many other tissues suggests that PKC plays an important role in the control of neuronal activity.12,23,24 In its resting state, PKC is present mainly in the cytosol. Stimulation of phosphoinositide C leads to formation of diacylglycerol and activation of PKC.23 The activation is associated with translocation of PKC activity from the cytosol to the membrane.23 Phorbol esters, such as PDBu, have a molecular structure similar to diacylglycerol. They mimic the effect of diacylglycerol by binding to the regulatory domain of PKC.12,23 In the present study PDBu, at concentrations that increased ARNA in WKY but not in SHR,11 produced a translocation of PKC from the cytosol to the membrane of the renal pelvic wall that was similar in WKY and SHR. These data suggest that the impaired responsiveness of renal sensory receptor activation by PDBu in SHR was not related to a defect in PKC activation in the renal pelvic wall but rather a defect in mechanisms distal to PKC activation.

Possible mechanisms involved in renal sensory receptor activation beyond activation of PKC include PGs and substance P. A role for PKC-mediated release of arachidonic acid in renal sensory receptor activation was suggested in our previous studies, which showed that renal pelvic administration of PDBu failed to increase ARNA in normotensive rats made arachidonic acid deficient by an essential fatty acid–deficient diet.15 Recent in vitro studies in cultured dorsal root ganglionic neurons have demonstrated that PDBu causes a calcium-mediated release of substance P from these sensory neurons.14 Our previous studies in normotensive rats demonstrated an important role for PGE and substance P in renal sensory receptor activation, the release of substance P being dependent on intact PG synthesis.5,10 Therefore we examined whether the renal pelvic release of PGE and/or substance P produced by increased renal pelvic pressure was altered in SHR. Similar to our previous studies in Sprague-Dawley rats,17 the increase in ARNA produced by increased renal pelvic pressure in WKY was associated with a reversible increase in the release of substance P and PGE into the pelvic effluent from the ipsilateral kidney. The amount of substance P released was related to the amount of PGE released (P<.01). In agreement with our previous studies in adult SHR,4,11 increasing renal pelvic pressure failed to increase ARNA in the majority of SHR. The impaired ARNA response was associated with decreased renal pelvic release of substance P. The increase in renal pelvic release of PGE produced by increased renal pelvic pressure was not different from that in WKY. These data suggest that the impaired responsiveness of renal pelvic sensory receptors in SHR is not related to a defect in the release of PGE produced by increased renal pelvic pressure but rather to an impaired release of substance P from renal sensory neurons.

The mechanisms involved in PG–mediated release of substance P are not known. It is conceivable that the increased release of PGE and substance P into the renal pelvic effluent during increased renal pelvic pressure is derived from the renal pelvic wall. We have previously shown that the isolated renal pelvis contains PGE and synthesizes PGE in response to mechanical stimuli.9 Likewise, our previous studies showed that the renal pelvic wall contains substance P.5 Furthermore, immunohistochemical studies have localized the majority of the renal sensory neurons containing substance P to the muscular layer of the renal pelvic wall.25 In vitro studies in cultured dorsal root ganglionic neurons have suggested that sensory neurons may be a source of PGs.26 PGE2 caused an enhancement of the bradykinin-mediated release of substance P that was dependent on extracellular Ca2+. Pretreating dorsal root ganglionic neurons with indomethacin blocked bradykinin-mediated release of substance P.26 These data suggest the interesting hypothesis that the sensory neurons may be able to autoregulate its release of neuropeptides by increasing and decreasing its PG synthesis. These studies are of interest in the context of the present study, showing similar PGE release in SHR and WKY but decreased substance P release in SHR in response to increased renal pelvic pressure. Moreover, previous studies have demonstrated impaired renal vasodilatory responses to PGE2 in SHR despite similar PGE2 receptor number as in WKY.27 The latter studies would suggest a defect in the PGE2 receptor–intracellular coupling mechanisms or beyond.
Our findings showing an impaired release of substance P in response to activation of renal pelvic sensory receptors in SHR are in analogy with previous studies in hypertensive rats demonstrating a failure of substance P to increase in response to feeding. Likewise, mental stress resulted in a reduced increase in the plasma concentration of substance P in hypertensive compared with normotensive subjects. In addition, several studies show an increased pain threshold in hypertensive men and rats. This is of interest in the context of the present study because substance P is known to be involved in mediating pain. These studies may indicate altered release of substance P and/or desensitization of substance P receptors as a possible mechanism involved in the increased pain threshold in hypertensive subjects.

Studies in nonneural vascular tissue would also indicate a defect at the level of the substance P receptors or beyond in hypertensive subjects. Substance P receptors have been localized in the renal pelvic area. In normotensive rats, renal pelvic administration of substance P results in a similar renal reflex response as increased renal pelvic pressure. Blockade of renal pelvic substance P receptors reduces the ARNA response to both increased renal pelvic pressure and bradykinin, suggesting that activation of renal pelvic substance P receptors contributes importantly to renal pelvic sensory receptor activation. We therefore hypothesized that impaired activation of renal pelvic substance P receptors might be an additional mechanism contributing to the decreased responsiveness of renal sensory receptors in SHR. Similar to our findings in Sprague-Dawley rats, renal pelvic administration of substance P increased ARNA in a dose-dependent fashion in WKY, the threshold of activation of renal pelvic substance P receptors being 0.8 µg/mL. However, in the majority of SHR, the responsiveness of the renal pelvic sensory receptors to substance P was markedly impaired. Likewise, these SHR did not respond to increased renal pelvic pressure. The number of SHR that responded to substance P was significantly less than the number of WKY at all concentrations of substance P. Whereas all WKY increased ARNA in response to substance P at 0.8 µg/mL and higher, the majority of SHR failed to increase ARNA above 0% in response to substance P at 0.8 to 20 µg/mL. Furthermore, in only 3 of 14 SHR did the administration of substance P at supramaximal concentration for activation of renal sensory receptors, 100 µg/mL (Reference 16 and the present study), produce an increase in ARNA in the range of that seen in WKY. The three rats that responded to substance P at 100 µg/mL did not differ in age or mean arterial pressure from the majority of SHR that showed impaired ARNA responses to substance P. In a minority of SHR (3 of 17 SHR), substance P at 4 to 100 µg/mL resulted in increases in ARNA that were similar to those in WKY. Because these SHR also responded to renal pelvic pressure with an increase in ARNA, these data provide further support for the hypothesis that the impaired ARNA response to increased renal pelvic pressure, seen in the majority of SHR, is related to impaired activation of substance P receptors.

Activation of substance P receptors contributes importantly to activation of renal sensory receptor activation by such seemingly different stimuli as increased renal pelvic pressure and bradykinin in normotensive rats. The results of the present studies would suggest that the impaired ARNA response to increased renal pelvic pressure and bradykinin in SHR is, at least in part, related to a decreased number of substance P–containing neurons and/or a defect in the release of substance P and/or desensitization of substance P receptors as a possible mechanism involved in the increased pain threshold in hypertensive subjects.

In summary, the results of the present study show that PDBu activated PKC in the renal pelvic wall to a similar extent in WKY and SHR. Increasing renal pelvic pressure increased ARNA in WKY but not in SHR. The increase in ARNA was associated with increased renal pelvic release of PGE and substance P in WKY. In SHR, the renal pelvic release of PGE was similar to that in WKY. However, the renal pelvic release of substance P was markedly suppressed. Furthermore, substance P increased ARNA in a concentration-dependent fashion in WKY, but in SHR the ARNA responses to substance P were impaired. These studies suggest that the impaired activation of renal sensory neurons in SHR is related to decreased release of substance P and/or decreased activation of substance P receptors. The increased activation of substance P receptors may be due to decreased number of substance P receptors or a defect beyond the substance P receptor. Substance P release from the renal sensory neurons may increase urinary sodium excretion via a direct effect on proximal tubules and by decreasing ERSNA through activation of the renorenal reflexes. Therefore one may speculate that the impaired renal release of substance P and activation of substance P receptors in SHR may contribute to the enhanced sodium retention observed in SHR.

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