Substance P in Subtotal Nephrectomy-Salt Hypertension

Khurshed A. Katki, Scott C. Supowit, Donald J. DiPette

Abstract—We have previously demonstrated that calcitonin gene-related peptide (CGRP) plays a counterregulatory role in subtotal nephrectomy-salt (SN-salt) hypertension through an increase in vascular responsiveness to the dilator activity of this neuropeptide. Substance P (SP) is often co-localized with CGRP in perivascular sensory nerves. To determine the role and mechanism of action of SP in SN-salt hypertension, we induced hypertension in 4- to 6-week-old male Sprague-Dawley rats (n = 8) by subtotal nephrectomy and 1% saline drinking water. Sham-operated rats were given either tap water (n = 9) or 1% saline to drink (n = 9). Eleven to 13 days after each protocol, all rats had intravenous (for drug administration) and arterial (for continuous monitoring of mean arterial pressure [MAP]) catheters surgically implanted and were studied in the conscious and unrestrained state. Baseline MAP was significantly elevated in the SN-salt rats (157 ± 6 mm Hg) compared with tap water–fed controls (128 ± 3 mm Hg) and 1% saline–fed controls (132 ± 5 mm Hg). Vehicle administration did not alter the MAP in any group. In contrast, administration of spantide-II (0.2 μmol/L in saline), an SP receptor antagonist, significantly elevated the MAP in SN-salt rats (13.9 ± 0.8 mm Hg) compared with the tap water (1.7 ± 1.7 mm Hg) and 1% saline controls (2.0 ± 1.9 mm Hg). SP mRNA and peptide levels in dorsal root ganglia were not significantly different between the 3 groups. Administration of exogenous SP (12 and 24 nmol · L⁻¹ · kg⁻¹ intravenously) resulted in a significantly greater decrease in MAP in the SN-salt rats compared with both control groups. Taken together, these data suggest that in SN-salt hypertension, SP plays a counterregulatory role in the absence of an increase in its neuronal expression, thereby suggesting that one possible mechanism of this compensatory vasodilator response is enhanced vascular reactivity to SP. (Hypertension. 2002;39[part 2]:389-393.)

Key Words: rats iii neuropeptides iii renal disease iii blood pressure iii RNA iii radioimmunoassay

Substance P (SP), an 11–amino acid neuropeptide, is a member of the tachykinin family that also includes neurokinin A (NK-A), neurokinin B (NK-B), neuropeptide K (NP-K), and neuropeptide-γ (NP-γ).¹² SP is derived from tissue-specific alternative splicing of the preprotachykinin I gene and is produced almost exclusively in neuronal tissues.² A prominent site for SP synthesis is the dorsal root ganglia (DRG), which contain the cell bodies of sensory neurons that terminate centrally in the spinal cord and peripherally on blood vessels.³ SP and its receptor (NK-1 receptor) are widely distributed in the central and peripheral nervous system. Immunoreactive substance P (iSP) containing nerve fibers are widely distributed in the brain as well as peripheral sensory nerve endings, where it is often co-localized and co-released with calcitonin gene-related peptide (CGRP).⁴ SP is involved in various physiological activities such as neuromodulation, smooth muscle contraction, and the modulation of vascular tone.⁵,⁶

A role for CGRP in experimental models of hypertension has been established. We have demonstrated that CGRP plays a counterregulatory role in deoxycorticosterone-salt (DOC-salt) hypertension as well as in subtotal nephrectomy-salt (SN-salt) hypertension.⁷⁸ Whereas in DOC-salt hypertension the antihypertensive activity of CGRP is mediated through an increase in neuronal expression and peptide release, in SN-salt hypertension this antihypertensive activity is mediated via enhanced sensitivity of the vasculature to the vasodilator activity of this peptide.⁹ Likewise, Kohlmann et al¹⁰ reported that endogenous SP acts to attenuate the blood pressure (BP) increase in 3 rat models of salt-dependent hypertension, including the SN-salt model. However, the status of SP expression, peptide release, as well as the mechanism of its counterregulatory role has not been determined in this setting. Therefore, the aims of this study were to confirm the depressor effect of endogenous SP using a peripherally acting peptide NK-1 receptor antagonist and to determine the status of neuronal expression and release of SP in SN-salt–induced hypertension. We also present a possible mechanism for the compensatory depressor action of this neuropeptide in this model of experimental hypertension.

Methods

Animals

All studies were approved by the Institutional Animal Care and Use Committee. A total of 26 male Sprague-Dawley rats (Harlan, Sprague Dawley, Indianapolis, Ind), 4 to 6 weeks old, were studied. For the surgical procedures, the rats were anesthetized with ketamine

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and xylazine (80 and 4 mg/kg intraperitoneally, respectively). SN-salt hypertension was induced in 8 animals by the total removal of the right kidney and approximately 25% of the left kidney (upper and lower poles). They received 1% saline to drink ad libitum. Sham-operated animals that received either tap water (n=9) or 1% saline (n=9) served as the controls. The tail-cuff method (Narco Bio-Systems) was used to record systolic BPs before the surgeries and on days 4, 7, and 10 thereafter. The SP antagonist studies were performed 11 to 13 days after the surgical procedures.

**SP Receptor Agonist/Antagonist Administration and Mean Arterial Pressure Measurement**

Substance P and spanptide-II (span-II), a potent peripherally acting peptide NK-1 receptor antagonist, were obtained from Phoenix Pharmaceuticals. Span-II has been previously shown to block the hypotensive effects of exogenously administered SP in normal rats. For the present studies, each animal was anesthetized as described above. The left carotid artery was cannulated for continuous monitoring of mean arterial pressure (MAP) using a pressure transducer coupled to a recorder (Gould Instruments). The right jugular vein was also cannulated for infusion of either vehicle or SP or the antagonist. The hemodynamic studies were performed approximately 3 hours after surgery with the rats fully awake and unrestrained.

**Hybridization Probes, RNA Isolation, and Analysis**

The SP probe was a 567-bp EcoRI-Hind III cDNA fragment of the rat β-preprotachykinin cRNA. The 18s rRNA hybridization probe was a 1.15-kb BamHI-EcoRI restriction fragment of the mouse 18s rRNA gene. The DNA inserts were purified by agarose-gel electrophoresis and subsequently labeled with \([α-32P]dCTP\) using a random hexanucleotide DNA labeling kit (Amersham Pharmacia Biotech).

After the hemodynamic studies, the rats were deeply anesthetized by infusion of ketamine and xylazine (100 and 5 mg/kg) through the jugular vein catheter. The rats were then euthanized, and DRG isolated by the guanidine-isothiocyanate method. Total cellular RNA was isolated by the guanidine-isothiocyanate method. RNA samples were size-separated by electrophoresis on denaturing formaldehyde-agarose gels. These fractionated RNAs were transferred onto a nylon membrane and hybridized with the \(32P\)-labeled SP probe. As shown in Figure 1, the systolic BP was rapidly and significantly elevated in the SN-salt rats (187±3 mm Hg; \(P<0.001\)) compared with tap water control rats (138±3 mm Hg) as well as the 1% saline control rats (145±3 mm Hg). Between days 11 and 13, all of the rats had arterial (for continuous MAP monitoring) and intravenous (for drug administration) catheters surgically implanted and were studied in the fully awake and unrestrained state. Baseline MAP was significantly elevated in the SN-salt rats (157±6 mm Hg; \(P<0.001\)) when compared with the tap water–fed sham-operated rats (128±3 mm Hg) as well as the 1% saline–fed sham-operated rats (132±5 mm Hg), respectively. Intravenous vehicle administration did not significantly alter the MAP in any of the groups (SN-salt rats, 3.8±0.5, versus tap water controls, 2.3±0.8, and 1% saline controls, 4.7±0.5 mm Hg). In contrast, as shown in Figure 2, intravenous administration of span-II (0.2 \(\mu\)mol/L in 0.1 mL of saline) resulted in a rapid increase of the already elevated MAP in the SN-salt hypertensive rats (13.9±0.8 mm Hg; \(P<0.001\)) when

![Figure 1. Systolic BP increases rapidly and significantly in SN-salt hypertension. The indirect tail-cuff method was used to record systolic BPs in SN-salt rats (n=8) and sham-operated controls that were given either tap water (n=9) or 1% saline (n=9) to drink. BPs were determined on days 0, 4, 7, and 10 of the study period. The values are reported as mean±SEM. ***P<0.001, SN-salt versus control-NS (tap water) and control-HS (1% saline).](image1)

![Figure 2. Span-II increases the MAP in SN-salt rats but not in control rats. Rats were instrumented for continuous MAP recording and span-II administration as described in the text. With the rats fully awake and unrestrained, bolus doses of the vehicle (0.1 mL of saline) and span-II (0.2 \(\mu\)mol/L in 0.1 mL) were administered intravenously. Values reported are change in MAP ±SEM (mm Hg) from baseline MAP. ***P<0.001 SN-salt versus control-NS (tap water) and control-HS (1% saline).](image2)

**Results**

**Hemodynamic Effects of Span-II**

As shown in Figure 1, the systolic BP was rapidly and significantly elevated in the SN-salt rats (187±3 mm Hg; \(P<0.001\)) when compared with tap water control rats (138±3 mm Hg) as well as the 1% saline control rats (145±3 mm Hg). Between
compared with the tap water (1.7±1.7 mm Hg) and the 1% saline control (2.0±1.9 mm Hg) groups. Furthermore, in our previous study on DOC-salt hypertension, uninephrectomized rats that were non–salt-loaded did not exhibit a significant change in MAP after span-II administration, when compared with sham-operated rats (3.6±0.4 mm Hg versus 0.9±2.1 mm Hg). The pressor activity of span-II was relatively short-lived; the MAP began to rise 30 s after a bolus dose of span-II and was elevated for approximately 210 s. The reason for this is likely that span-II, a peptide antagonist, is rapidly degraded in the circulation. As part of these experiments, we also measured the heart rate, which was not significantly changed by span-II in any of the groups. These results demonstrate that in SN-salt–induced hypertension, SP plays a compensatory vasodilator role to buffer the increase in BP.

Analysis of SP mRNA and iSP Content in SN-Salt Hypertensive and Control Rats

To determine whether neuronal SP expression was altered in SN-salt hypertension, we quantified the mRNA and immunoreactive peptide levels in DRG taken from the animals used in the experiments described above. Figure 3 is a representative Northern blot showing the 567-bp SP mRNA and the 18S rRNA present in the DRG RNA samples of tap water control and SN-salt rats. Scanning densitometry was performed to quantify the hybridization signals for each RNA species. To control for possible differences in loading of the RNA samples, we normalized the mRNA levels to those for 18S rRNA. Figure 4 depicts the SP mRNA levels from DRG. To determine whether there is an increased sensitivity of the vasculature to exogenously administered SP in vivo. Three groups (n=6/group) of rats, identical to those described above, were intravenously administered saline (0.1 mL) followed by 12 nmol · L⁻¹ · kg⁻¹ SP, and their MAPs were recorded. Baseline MAPs were similar to those obtained previously. In another experiment, an additional 3 groups of animals were administered saline (0.1 mL) and 24 nmol · L⁻¹ · kg⁻¹ SP. As seen in Figure 5, the decrease in baseline MAP in the SN-salt animals was not significantly different from the tap water controls 0.13±0.01; 1% saline controls 0.27±0.03 arbitrary units). The reason for this, however, is not known.

Levels of iSP in the DRG were measured by radioimmunoassay, the results of which are also shown in Figure 4. At the end of the study period, iSP levels were not significantly different among the study groups (SN-salt 0.15±0.01; tap water controls 0.13±0.01; 1% saline controls 0.11±0.04 pg iSP/µg total protein).

Effect of SP on MAP

We have previously reported that the neuronal expression and release of CGRP is unaltered in SN-salt hypertension and have demonstrated increased sensitivity of the vasculature to this peptide in this setting. Our results with neuronal SP expression and release in SN-salt hypertension were similar to those we obtained for CGRP. To determine whether there is an increased sensitivity of the vasculature to SP in this model, we studied the response of the MAP to different doses of SP. Figure 5 shows a dose-dependent BP response to exogenously administered SP in vivo. Three groups (n=6/group) of rats, identical to those described above, were intravenously administered saline (0.1 mL) followed by 12 nmol · L⁻¹ · kg⁻¹ SP, and their MAPs were recorded. Baseline MAPs were similar to those obtained previously. In another experiment, an additional 3 groups of animals were administered saline (0.1 mL) and 24 nmol · L⁻¹ · kg⁻¹ SP. As seen in Figure 5, the decrease in baseline MAP in the SN-salt animals...
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The results presented herein are consistent with the findings of Kohlmann et al, who showed that acute inhibition of the NK-1 receptor with a non-peptide NK-1 receptor antagonist that is known to penetrate the central nervous system (CNS) and generate a delayed and sustained pressor response. Because the pressor effects of non-peptide NK-1 receptor antagonists may be partially mediated through the CNS as well as at the level of vascular NK-1 receptors, we decided to evaluate the role of SP in the same setting using span-II, a potent peptide NK-1 receptor antagonist. On administration of span-II, there is a rapid and short-lived pressor response to the already elevated MAP of SN-salt hypertensive rats. Correspondingly, no significant change in MAP was observed in the tap water control animals. Because this antagonist probably does not penetrate the CNS, it is therefore likely that the pressor response is primarily due to span-II blocking peripheral vascular NK-1 receptors. This hypertensive effect of span-II is not the result of salt-loading alone, because in sham-operated 1% saline control animals, no significant rise in MAP was observed after antagonist administration. However, it is possible that the effect of SP on BP is secondary to the subtotal nephrectomy. This is unlikely as in our previous studies on DOC-salt hypertension, control animals that underwent uninephrectomy did not exhibit a pressor response on intravenous administration of span-II.  

The results presented herein are consistent with the findings of Kohlmann et al, who showed that acute inhibition of the NK-1 receptor with a non-peptide SP antagonist increased the MAP in 3 salt-dependent models, DOC-salt, SN-salt, and 1-kidney-1 clip, but failed to do so in genetic and salt-independent models such as the spontaneously hypertensive rats (SHR) and the 2-kidney-1 clip model. We have since extended these results to the DOC-salt model of hypertension, in which the neuronal expression and release of SP is increased as a compensatory mechanism to the elevated BP, and in the DAH-salt model (a genetic and salt-independent model of hypertension), in which SP does not seem to play a compensatory vasodilator role. In addition, these data are in keeping with reports from the literature, which indicate reduced plasma levels of SP observed in the SHR and human essential hypertension and the elevation of BP in these models could be due, at least in part, to the insufficient synthesis and release of endogenous SP.

To determine the status of neuronal SP production and release, we quantified SP mRNA and iSP content from DRG of the animals used in the above experiments. We found that there was no change in the neuronal SP expression and peptide levels in SN-salt hypertension when compared with both the control groups of animals. This is similar to our previous studies evaluating the role of CGRP in SN-salt and pregnant hypertensive rats, in which endogenous CGRP was shown to play a counterregulatory role and neuronal expression and release of CGRP was unaltered.

In the absence of any increase in neuronal SP expression, we decided to explore the mechanism of the counterregulatory role of this peptide in SN-salt hypertension. One possible mechanism to explain this depressor effect of SP in SN-salt hypertension is an increased vascular sensitivity to this peptide. Indeed, when exogenous SP is administered to SN-salt rats, the antihypertensive effect of SP is more pronounced in SN-salt rats than in both groups of control rats as shown in Figure 5. This significant decrease is maintained when corrected for baseline MAP. Hence, these data demonstrate that one mechanism of the counterregulatory effect of SP in this setting is through an increased vascular reactivity to this peptide.

In summary, using a peptide NK-1 receptor antagonist, we have confirmed that substance P plays a counterregulatory role in SN-salt hypertension to partially counteract the elevated BP in this model of hypertension in the absence of any detectable change in its neuronal expression and release. These data also indicate that the pressor effect of span-II in this setting is due mainly to the blocking of vascular NK-1 receptors by the antagonist. The antihypertensive role of SP in this setting is similar to its role in DOC-salt and the 1-kidney-1 clip model of salt-dependent hypertension. We have also shown that one possible mechanism of action of SP in SN-salt hypertension is the increased vascular reactivity to this peptide as opposed to enhanced neuronal expression. Additional studies, however, need to be performed to evaluate this mechanism.

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