Role Of Calcitonin Gene-Related Peptide and Substance P in Dahl-Salt Hypertension

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Abstract—Calcitonin gene-related peptide (CGRP) and substance P are known to play a counterregulatory role in acquired models of salt-dependent hypertension. In contrast, neuronal production of these peptides is decreased in the spontaneously hypertensive rat, which may contribute to the elevated blood pressure. To determine the role played by CGRP and substance P in Dahl-salt hypertension, 4- to 6-week-old male salt-resistant (DR) and salt-sensitive (DS) rats were divided into 4 groups (n=5/group) and pair-fed low-salt (0.2% NaCl) (DR/LS and DS/LS) and high-salt (8% NaCl) diets (DR/HS and DS/HS) for 3 weeks. After 3 weeks, all the rats had venous (for drug administration) and arterial (for blood pressure monitoring) catheters surgically implanted and were studied in the conscious and unrestrained state. Mean arterial pressure was significantly higher in the DS/HS rats (185.8±1.6 mm Hg, P<0.001) compared with DR/LS rats (145.8±1.6 mm Hg, P=0.01). Intravenous administration of CGRP and SP receptor antagonists was without effect in any of the groups studied. CGRP and SP mRNA content from dorsal root ganglia were not significantly different between the groups. Whereas immunoreactive CGRP was decreased in the DS groups (DS/HS, 9.4±0.4 pg/μg protein; DS/LS, 11.1±0.8 pg/μg protein; P<0.01) compared with the DR groups (DR/HS, 13.9±0.6 pg/μg protein; DR/LS, 14.6±0.6 pg/μg protein), neuronal SP production was similar between all the groups. Thus, CGRP and substance P do not play a counterregulatory role in Dahl-salt hypertension. The decrease in neuronal CGRP expression in DS rats appears to be genetically determined as in SHR, however, and may contribute to the increase in blood pressure following salt-loading. (Hypertension. 2001; 38[part 2]:679-682.)

Key Words: rats ■ neuropeptides ■ hypertension, genetic ■ blood pressure ■ RNA ■ radioimmunoassay

Calcitonin gene-related peptide (CGRP), a 37 amino acid peptide, is produced by the alternate splicing of the calcitonin/CGRP gene.1,2 CGRP is the most potent vasodilator discovered to date and has markedly positive chronotropic and inotropic effects.3 There are 2 CGRP genes, which are called α-CGRP and β-CGRP in the rat and I and II humans.3 In the rat and in humans, the 2 peptides differ by 1 amino acid and 3 amino acids, respectively, and their biological activities are similar in most vascular beds. Substance P (SP) is a member of the tachykinin family and, like CGRP, is involved in numerous physiological activities such as neuromodulation, smooth muscle contraction, and vasodilation.4,5 A prominent site for CGRP and 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.5

We have demonstrated that the neuronal expression of CGRP is decreased in spontaneously hypertensive rats (SHR) compared with Wistar-Kyoto rats (WKY).6 Also, reduced levels of SP have been reported in essential hypertension in humans and in stroke-prone SHR.7,8 Therefore, in these settings both CGRP and SP could contribute to elevated blood pressure through the decreased activity of a potent vasodilator. In contrast, we have reported that CGRP plays a counterregulatory role in deoxycorticosterone acetate (DOCA)-salt hypertension and in subtotal nephrectomy–salt (SN-salt) hypertension.9,10 Whereas in DOCA-salt hypertension the counterregulatory effect of CGRP is mediated through increased neuronal expression (and peptide release), in SN-salt hypertension this antihypertensive activity is mediated via the enhanced sensitivity of the vasculature to the vasodilator activity of this peptide.11 Recently, we have determined that these same mechanisms may mediate the antihypertensive effects of SP in the DOCA-salt and SN-salt models (K.A. Katki, S.C. Supowit, D.J. DiPette, unpublished observations, 2001). However, the role played by these neuropeptides in the hypertensive process in the Dahl rats is unknown. The aim of this study was, therefore, to determine the role of CGRP and SP in Dahl-salt hypertension.

Methods

Animals
All studies were approved by the Institutional Animal Care and Use Committee. A total of 20 male Dahl salt-resistant (DR) and Dahl salt-sensitive (DS) animals, 4 to 6 weeks old, were divided into 4 groups (5 per group) and pair-fed low-salt (0.2% NaCl) (DR/LS and...
CGRP and SP Receptor Antagonist
Administration and MAP Measurement
Rat α-CGRP,35 and Spantide-II (Span-II) were obtained from Phoenix Pharmaceuticals. CGRP135 was dissolved in saline and has previously been shown to block the hypotensive effects of intravenously infused rat α-CGRP in normal rats.9 Span-II, a potent peptide NK-1 receptor antagonist, was dissolved in dimethyl sulfoxide (J.T. Baker), and the volume was made up with saline. Each rat was anesthetized with ketamine and xylazine (80 and 4 mg/kg IP, respectively). The left carotid artery was cannulated for continuous monitoring of mean arterial pressure (MAP) by a pressure transducer coupled to a recorder (Gould Instruments). The right jugular vein was also cannulated for infusion of either vehicle or the antagonists. The hemodynamic studies were performed ∼3 hours after surgery, with the rats fully awake and unrestrained.

Hybridization Probes, RNA Isolation, and Analysis
The α-CGRP hybridization probe was a 1.4-kb Sau 3A restriction fragment spanning exons 5 and 6 of the rat CGRP gene.12 The SP probe was a 567-bp EcoRI-Hind III cDNA fragment of the rat β-propreotachykinin cRNA.13 The 18S rRNA hybridization probe was a 1.15-kb BamHI-EcoRI restriction fragment of the mouse 18S rRNA gene.14 The DNA inserts were purified by agarose-gel electrophoresis and subsequently labeled with [α-32P]dCTP by a random hexanucleotide DNA labeling kit (Amersham Pharmacia Biotech).

Radioimmunoassay
To determine immunoreactive CGRP (iCGRP) and immunoreactive SP (iSP) from DRG, we used commercially available rabbit anti-rat CGRP and SP RIA kits (Phoenix Pharmaceuticals). The antibody for CGRP has 100% reactivity with rat α-CGRP and 79% reactivity with β-CGRP. There is no cross-reactivity with amylin, calcitonin, somatostatin, or SP. The antibody for SP has 100% reactivity with rat SP. There is no cross-reactivity with neurokinin A (NK-A), neurokinin B (NK-B), somatostatin, or physalaemin. The assay was performed as recommended by the supplier, and total protein was determined by method of Bradford (Bio Rad).

Statistical Analysis
Statistical significance was determined by ANOVA followed by the Tukey-Kramer multiple comparisons test. The acceptable level of significance was P<0.05. Data in the figures are represented as mean±SEM.

Results
Determination of the Dose of NK1 Receptor Antagonist (Span-II)
To determine whether Span-II could block the hypotensive effects of exogenous SP in normal rats, Sprague-Dawley rats (weight, 250 grams; n=3) were anesthetized and instrumented for continuous MAP recording and drug administration. When the rats were fully awake and unrestrained, they were injected with bolus doses of SP (6 and 12 nmol·kg⁻¹·min⁻¹) alone and a mixture of SP and Span-II. Bolus doses of Span-II (0.2 μmol/L and 0.4 μmol/L) alone had no effect on the MAP; however, when SP and Span-II were given sequentially (either SP or Span-II first), the antagonist was able to completely inhibit any reduction in MAP (data not shown). These results indicate that Span-II blocks the hypotensive effect of exogenously administered SP.

Regulatory Effects of CGRP8-37 and Span-II
After 3 weeks, the systolic BP was significantly elevated in DS/HS animals (185.8±1.6 mm Hg, P<0.001) compared with the DS/LS (149.3±2.7 mm Hg), DR/LS (150.2±0.6 mm Hg), and DR/HS (148.8±0.8 mm Hg) groups. All the rats then 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 DS/HS rats (177±4.9 mm Hg, P<0.01) compared with the DS/LS (129.3±1.8 mm Hg), DR/LS (132.3±7.9 mm Hg), and DR/HS groups (147.3±3 mm Hg), as shown in Figure 1. Vehicle administration did not significantly alter the MAP in any of the groups. Intravenous administration of CGRP8-37, and Span-II was also without effect in any of groups studied.

Analysis of CGRP and SP mRNA and Peptide Levels in Dahl-Salt Rats
To determine whether neuronal CGRP and/or SP expression was altered in any of the 4 groups of rats, we quantified the

Figure 1. MAP is significantly elevated in the DS/HS rats. At the end of the 3-week treatment period, the rats were instrumented for continuous MAP recording and intravenous drug administration as described in the text. ***P<0.001 DS/HS vs DS/LS, DR/HS, and DR/LS.
mRNA and immunoreactive peptide levels in DRG taken from the animals used in the experiments described above. Figure 2 is a representative Northern blot showing the 1.2-kb CGRP mRNA species, the 567-bp SP mRNA, and the 18S rRNA present in the DRG RNA samples. 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 3 depicts the mRNA levels of both neuropeptides studied. Although CGRP mRNA content from DRG of DS rats was somewhat lower, this reduction was not significantly different from the other groups (DS/HS, 0.46 ± 0.03; DS/LS 0.57 ± 0.03; DR/LS, 0.52 ± 0.03; DR/HS, 0.56 ± 0.04 arbitrary units). Similarly, the SP mRNA content did not differ between the 4 groups (DS/HS, 0.29 ± 0.01; DS/LS, 0.24 ± 0.02; DR/LS, 0.24 ± 0.02; DR/HS, 0.27 ± 0.03 arbitrary units).

Levels of iCGRP and iSP in the DRG were measured by radioimmunoassay, the results of which are shown in Figure 4. At the end of the study period, iCGRP levels were decreased in the DS rats (DS/HS, 9.4 ± 0.3 pg/mg protein; DS/LS, 10.1 ± 0.3 pg/mg protein; both P < 0.01) compared with the DR rats (DR/LS, 12.9 ± 0.4 pg/mg protein; DR/HS, 10.9 ± 0.6 pg/mg protein). Immunoreactive SP, however, was not significantly different among the study groups (DS/HS, 13.2 ± 0.6 pg/100 mg protein; DS/LS, 13.3 ± 0.3 pg/100 mg protein; DR/LS, 11.8 ± 0.6 pg/100 mg protein; and DR/HS, 11.9 ± 0.5 pg/100 mg protein).

**Discussion**

This study was undertaken to evaluate the role of CGRP and SP in Dahl-salt hypertension. Previously, we have shown in DOCA-salt hypertension, SN-salt hypertension, and nitro-L-arginine methyl ester (L-NAME)–induced hypertension during pregnancy9,10,16 that the acute administration of CGRP8-37 increases the MAP, thereby suggesting a counterregulatory role for this peptide in these models of experimental hypertension. A compensatory vasodilator role for SP in 3 models of salt-dependent hypertension—DOCA-salt, SN-salt, and the 2-kidney, 1-clip model—has also been reported.7 We have since confirmed and extended these results in the DOCA-salt and SN-salt models (K.A. Katki, S.C. Supowit, D.J. DiPette, unpublished observations, 2001). In contrast, acute administration of either the CGRP or the SP receptor antagonist to the Dahl-salt rats did not significantly increase the MAP in any of the groups studied. Therefore, these data suggest that in this setting, these sensory neuropeptides do not act as compensatory vasodilators to attenuate the BP increase as seen in the aforementioned acquired models of hypertension. Indeed, the results described herein are consistent with the observations made with SHR. We have reported that acute CGRP8-37 administration does not increase the MAP in this purely genetic model of hypertension, whereas Kohlmann et al7 obtained similar results following infusion of a nonpeptide NK-1 receptor antagonist.

To evaluate CGRP and SP mRNA and peptide levels, it was necessary to study DRG, because they are the site of sensory neuron cell bodies that terminate peripherally on blood vessels and centrally in laminae I/II of the dorsal horn in the spinal cord. Several lines of evidence indicate that the
vasodilator actions of CGRP and SP are caused by the efferent release of these peptides from perivascular sensory nerve terminals. It is thought that circulating CGRP (and SP) are largely derived from these perivascular nerve terminals and represent a spillover phenomenon related to the release of these peptides to promote vasodilation or other functions. What roles circulating CGRP and SP might play remains controversial. Currently, it is not known whether reuptake of CGRP and SP plays a significant role in regulating the biological activation of these neuropeptides. The present results demonstrate that CGRP mRNA accumulation is not different between the study groups. Unexpectedly, iCGRP levels in both DS/HS and DS/LS rats were significantly decreased (30% and 22%, respectively) compared with the levels in DR/LS rats. The reason for this discrepancy between CGRP mRNA and peptide content is unclear. This finding in the Dahl-salt model is similar to observations made in SHR, in which there is also a decrease in neuronal CGRP expression. In regards to the latter model, we have demonstrated that the reduction in CGRP production may contribute to the elevated BP. Likewise, in the DS rats the decrease in neuronal CGRP may be genetically determined and possibly contribute to the increase in BP following a high-salt diet. There was no detectable change in either SP mRNA or peptide expression in the DRG. We have previously shown that the isSP content in the spinal cord is not different between SHR and WKY controls; however, other investigators have demonstrated reduced circulating levels of this peptide in the SHR and stroke-prone SHR. Therefore, although it is possible to speculate that the decreased activity of SP may contribute to the BP increase in the SHR, this does not appear to be the case in the Dahl-salt rats.

In summary, the novel feature of this study is that Dahl-salt hypertension appears to be unique from other models like DOCA-salt and SN-salt hypertension, in that neuronal CGRP and SP do not appear to serve counterregulatory roles in this specific form of salt-dependent hypertension. In addition, although speculative, the reduction in CGRP expression in the DS rats may be genetically determined and possibly contribute to the salt-induced increase in BP.

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References
