CGRP Activates Renal Pelvic Substance P Receptors by Retarding Substance P Metabolism

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Abstract—Substance P and calcitonin gene–related peptide (CGRP) are colocalized in renal pelvic sensory nerves. Increasing renal pelvic pressure results in an increase in afferent renal nerve activity that is blocked by a substance P receptor antagonist but not by a CGRP receptor antagonist. CGRP potentiates the effects of substance P by preventing the metabolism of substance P. Therefore, we examined whether CGRP enhanced the afferent renal nerve activity responses to substance P and increased renal pelvic pressure, a stimulus known to increase substance P release. Combined administration of substance P and CGRP into the renal pelvis resulted in an increase in afferent renal nerve activity (1392±217% · s; area under the curve of afferent renal nerve activity versus time) that was greater (P<0.01) than that produced by substance P (620±156% · s) or CGRP (297±96% · s) alone. Likewise, CGRP enhanced the afferent renal nerve activity response to increased renal pelvic pressure. During renal pelvic administration of the neutral endopeptidase inhibitor thiorphan, the afferent renal nerve activity response to substance P plus CGRP was similar to that produced by either neuropeptide alone. Because these studies suggested that CGRP potentiated the afferent renal nerve activity responses to substance P, we examined whether the afferent renal nerve activity response to CGRP was blocked by a substance P receptor antagonist, RP67580. RP67580 blocked the afferent renal nerve activity response to CGRP by 85±12% (P<0.02). We conclude that CGRP activates renal pelvic sensory nerves by retarding the metabolism of substance P, thereby increasing the amount of substance P available for stimulation of substance P receptors. (Hypertension. 1999;33[part II]:493-498.)

Key Words: sensory neurons  ■ endopeptidase, neutral  ■ afferent renal nerve

Obstruction to urine flow increases renal pelvic pressure and activates renal mechanosensitive neurons, resulting in an increase in ipsilateral afferent renal nerve activity (ARNA).1–4 The increase in ARNA produces a decrease in contralateral afferent renal nerve activity and a contralateral diuresis and natriuresis, known as the contralateral inhibitory renorenal reflex.

The mechanosensitive neurons activated in this reflex are mainly located in the renal pelvic wall.5,6 Activation of substance P receptors in the renal pelvic area plays an essential role in the activation of renal mechanosensitive neurons.2–4 The renal pelvic sensory neurons contain substance P, and increasing renal pelvic pressure increases renal pelvic release of substance P.3,4 Similar to sensory neurons in other tissues, the renal sensory neurons also contain calcitonin gene-related peptide (CGRP), with substance P and CGRP being colocalized in many neurons.5,6 Administration of CGRP into the renal pelvis results in an increase in ARNA that is blocked by a CGRP receptor antagonist, suggesting the presence of CGRP receptors in the renal pelvic area.7 However, blocking these receptors has no effect on the increase in ARNA produced by increased renal pelvic pressure.7 This was somewhat unexpected considering that the CGRP-containing neurons appear to be more abundant than the substance P–containing neurons in the renal pelvic wall.5,6

The present study was performed to examine the role of CGRP in the activation of renal sensory neurons. The colocalization of substance P and CGRP in many central and peripheral sensory neurons suggests a functional interaction between the 2 neuropeptides.8 Numerous studies have shown that CGRP potentiates the effect of substance P, thus serving as a neuromodulator.8 To date, most of these studies concern central interaction between the 2 neuropeptides. For example, CGRP potentiates the effects of substance P on the spontaneous and noxious evoked activity of dorsal horn cells9 and the biting-scratching behavior elicited by intrathecal administration of substance P.10 The mechanisms by which CGRP potentiates the effects of substance P have been explained by the competition of CGRP and substance P for the same catabolic enzyme, neutral endopeptidase (NEP).8,11,12 Also, CGRP has been shown to enhance the release of substance P from primary afferent neurons in the presence of NEP inhibitors.13
To investigate whether CGRP served as a neuromodulator of the effects of substance P on renal sensory nerves, we examined whether CGRP enhanced the ARNA response to exogenous administration of substance P and increased renal pelvic pressure, a stimuli known to release substance P.4 Because our results showed that CGRP potentiated the ARNA response to both exogenous and endogenously released substance P, we then examined whether inhibition of NEP would prevent the CGRP-mediated enhancement of the ARNA response to substance P and whether the ARNA response to CGRP per se would be blocked by a substance P receptor antagonist.

Methods
All animal procedures were performed in accordance with the guidelines of the University of Iowa Animal Care and Use Committee.

The study was performed on male Sprague-Dawley rats weighing 255 to 415 g (mean weight, 325±4 g). Anesthesia was induced with pentobarbital sodium (0.2 mmol/kg IP, Abbott Laboratories) and maintained with an infusion of pentobarbital sodium (0.04 mmol/kg per hour IV at 50 μL/min) into the femoral vein. Arterial pressure was recorded from a catheter in the femoral artery. The procedures for stimulating and recording ARNA have been previously described in detail.1,4 In short, the left kidney was approached through a flank incision, a PE-10 catheter was placed in the right ureter for collection of urine, and a PE-60 catheter was placed in the left ureter with its tip in the pelvis. A PE-10 catheter was placed inside the PE-60 catheter for administration of vehicle and various experimental drugs into the renal pelvis. ARNA was stimulated by increasing renal pelvic pressure or administering substance P and CGRP into the renal pelvis. Renal pelvic pressure was increased by elevating the PE-60 ureteral catheter above the level of the kidney. ARNA was recorded from the peripheral portion of the cut end of the renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-second intervals, the unit of measure being micro-volts per second per 1 second. 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.1,4

Experimental Protocols
Approximately 90 minutes elapsed between 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. Substance P and CGRP were administered into the renal pelvis over a period of 2.5 minutes at 20 μL/min.

Effects of CGRP on ARNA Response to Substance P
The experiment consisted of 3 parts separated by 10-minute intervals. Each part consisted of a 10-minute control, a 5-minute experimental, and a 10-minute recovery period. During each experimental period, either substance P, CGRP, or substance P+CGRP was administered into the renal pelvis in random order. Two groups were studied. CGRP was given at 0.026 μmol/L, and CGRP was administered into the renal pelvis in random order, the concentrations of substance P and CGRP being 3.7 and 0.026 μmol/L, respectively. Two groups were studied. In the first group (n=10), the renal pelvis was perfused at 20 μL/min with 0.1% ethanol (vehicle) during the first 3 parts and with the NEP inhibitor thiorphan at 10 μmol/L during the last 3 parts. In the second group (time control, n=9), the renal pelvis was perfused with 0.1% ethanol during all 6 parts.

Effects of a Substance P Receptor Antagonist on ARNA Responses to CGRP
The experiment consisted of 2 parts separated by a 20-minute interval. Each part consisted of a 10-minute control, 5-minute experimental, and 10-minute recovery period. During each experimental period, CGRP at 0.26 μmol/L was administered into the renal pelvis. The renal pelvis was perfused at 20 μL/min throughout the experiment with either vehicle (0.0005 N HCl), the substance P receptor antagonist RP67580, or its inactive enantiomer RP68651. Two groups were studied. In the first group (n=7), the renal pelvis was perfused with vehicle during the first part and with RP67580 (0.11 mmol/L) during the second part of the experiment. The renal pelvic perfusate was switched immediately after the first recovery period. The renal pelvis was perfused with the substance P receptor antagonist for 30 minutes before CGRP was administered during the second part of the experiment. In the second group (n=7), the renal pelvis was perfused with the inactive enantiomer RP68651 (0.11 mmol/L) during the second part of the experiment.

Drugs
Substance P and CGRP were dissolved in 0.15 mol/L NaCl. Thiorphan was dissolved in 100% ethanol and further diluted in 0.15 mol/L NaCl; final ethanol concentration was 0.1%. RP67580 and RP68651 were dissolved in 0.0005 N HCl. RP67580 and RP68651 were provided by Rhône-Poulenc Rorer Recherche- Développement, Vitry Sur Seine, France. Substance P, CGRP, and thiorphan were purchased from Sigma Chemical Co.

Statistical Analysis
Mean arterial pressure was measured continuously and averaged over each period. The effects of substance P and CGRP varied both in amplitude and duration during the various experimental conditions. Therefore, the ARNA responses to substance P and CGRP were calculated as the area under the curve of ARNA versus time (AUC), where ARNA was expressed as a percentage of its baseline value during the control period preceding each experimental period. Friedman 2-way ANOVA with multiple comparisons between groups, Mann-Whitney U test, and Wilcoxon matched-pairs signed-rank test were used.16 A significance level of 5% was chosen. Data in text and figures are expressed as mean±SE.

Results
Effects of CGRP on ARNA Response to Substance P
CGRP is colocalized with substance P in many sensory nerves, including the renal sensory nerves.5,6,8 CGRP is
known to potentiate the activation of central sensory neurons by substance P. We tested the idea that CGRP may also enhance the substance P-mediated activation of renal sensory nerves. Renal pelvic administration of substance P and CGRP resulted in significant increases in ARNA when administered separately (Figure 1). The ARNA response to 0.26 μmol/L CGRP (Figure 1B) was greater than that produced by 0.026 μmol/L CGRP (P<0.01, Figure 1A). In both groups, the ARNA response to the combined administration of substance P and CGRP resulted in an increase in ARNA that was greater than that produced by either neuropeptide alone (P<0.01). Furthermore, the ARNA response to the combined administration of substance P+CGRP was greater than the sum of the ARNA responses to substance P and CGRP when administered separately in either group (P<0.05). The greater ARNA response to the combined administration of substance P and CGRP was due largely to a prolongation of the response. The durations of the ARNA responses to substance P, 0.026 μmol/L CGRP, and substance P+CGRP were 35±6, 17±4, and 60±10 seconds, respectively. The ARNA response to substance P was unaltered throughout the course of the experiment in the 2 groups (25±5, 24±3, and 69±11 seconds, respectively).

**Effects of CGRP on ARNA Response to Increased Renal Pelvic Pressure**

Increasing renal pelvic pressure increases renal release of substance P. Activation of substance P receptors contributes importantly to the ARNA response to increased pelvic pressure. We reasoned that if the greater ARNA response to the combined administration of substance P and CGRP (Figure 1) was related to CGRP increasing the availability of substance P, then CGRP would enhance the ARNA response to increased renal pelvic pressure. The results are shown in Figure 2A. Increasing renal pelvic pressure by 10 mm Hg resulted in a reversible increase in ARNA. Renal pelvic administration of 0.026 μmol/L CGRP resulted in an increase in basal ARNA of 1222±360%·s (P<0.01) that lasted 43±11 seconds. Basal ARNA had returned to baseline before renal pelvic pressure was increased a second time. Increasing renal pelvic pressure after CGRP administration resulted in an ARNA response that was greater than that produced by increased renal pelvic pressure before CGRP (P<0.05, Figure 2A). In the time control experiments, increasing renal pelvic pressure twice in the absence of CGRP resulted in reproducible increases in ARNA (Figure 2B). Basal ARNA was unaltered throughout the course of the experiment in the 2 groups (607±73 to 594±63 and 810±221 to 764±229 μV·s/1 s, respectively). Mean arterial pressure, 102±6 and 93±2 mm Hg, was unaffected by increased renal pelvic pressure and remained unaltered throughout the experiments in the 2 groups.

**Effects of Thiorphan on ARNA Responses to Substance P and CGRP**

CGRP has been shown to potentiate substance P-induced activation of sensory nerves by retarding the metabolism of substance P by competing for the same catabolic enzyme, NEP, and/or enhancing the release of substance P. To examine whether CGRP enhanced the ARNA response to substance P by prolonging the breakdown of substance P, we compared the effects of the combined administration of substance P+CGRP in the absence and presence of the NEP inhibitor thiorphan. The results are shown in Figures 3 and 4. Similar to our previous studies (Figure 1), in the absence of thiorphan the combined administration of substance P+CGRP (0.026 μmol/L) resulted in an increase in ARNA that was greater than that produced by either substance P or CGRP alone (P<0.01, Figure 3A). However, in the presence of renal pelvic perfusion with thiorphan, the ARNA response to substance P+CGRP was not different from that produced by either substance P or CGRP when administered separately.
In the time control experiments, ie, in the absence of thiorphan, the ARNA responses to substance P, CGRP, and substance P+CGRP were reproducible (Figure 4A versus 4B). Basal ARNA did not change throughout the course of the experiment in the 2 groups (872 ± 6 to 923 ± 71 and 823 ± 63 to 872 ± 115 μV · s/1 s, respectively). Mean arterial pressure, 118 ± 2 and 118 ± 5 mm Hg, was unaltered throughout the experiment in the 2 groups.

Effects of a Substance P Receptor Antagonist on ARNA Responses to CGRP

We reasoned that if the activation of renal sensory nerves by CGRP was related at least in part to increased levels of substance P, the ARNA response to substance P, CGRP, and substance P+CGRP were decreased by a substance P receptor antagonist. The results are shown in Figure 5. The ARNA response to 0.26 μmol/L CGRP was blocked by 85 ± 12% (P < 0.02) by renal pelvic perfusion with the substance P receptor antagonist RP67580 (Figure 5A) administered at a concentration of 0.11 mmol/L, which we have previously shown blocks the ARNA response to substance P.1 RP68651, the inactive enantiomer of RP67580, had no effect on the ARNA response to CGRP (Figure 5B). Basal ARNA was unchanged throughout the course of the experiment in the 2 groups (1181 ± 138 to 1177 ± 155 and 1062 ± 41 to 947 ± 65 μV · s/1 s, respectively). Mean arterial pressure, 117 ± 4 and 114 ± 2 mm Hg, was not affected by CGRP or the SP receptor antagonist and remained unaltered throughout the experiment.

Discussion

These experiments show a synergism between substance P and CGRP in the activation of renal pelvic sensory receptors. The synergism between the 2 neuropeptides was absent in the presence of an NEP inhibitor. Furthermore, the CGRP-mediated activation of renal pelvic sensory neurons was blocked by a substance P receptor antagonist. Taken together, these results suggest that CGRP increases the availability of substance P for activation of renal sensory neurons.

Synergy Between Substance P and CGRP

Whereas there is ample evidence for an important role for substance P in the activation of renal mechanosensitive neurons,2–4 the role of CGRP is unclear. Our previous studies showed that the ARNA response to increased renal pelvic pressure was unaffected by a CGRP receptor antagonist.7 We were puzzled by these findings since the CGRP-containing neurons are at least as abundant, if not more so, than the substance P–containing neurons in the renal pelvic wall.5,6 However, there are numerous studies showing that CGRP enhances the effects of substance P at the spinal level.9–11,13 Therefore, we postulated that CGRP may have a similar potentiating effect on the substance P–mediated activation of renal pelvic sensory nerves. Our studies confirmed our hypothesis. Combined administration of substance P+CGRP into the renal pelvis resulted in an increase in ARNA that was greater than that produced by either neuropeptide, and the effects of substance P and CGRP were synergistic. Further-
more, CGRP potentiated the ARNA response to physiological activation of the renal sensory nerves produced by increased renal pelvic pressure, a stimulus known to be mediated by substance P.5,6,7 Taken together, these data suggest that CGRP enhances the activation of renal sensory nerves activated by either exogenous substance P or endogenously released substance P.

Interaction Between Substance P and CGRP: Role of NEP

The increased ARNA response to the combined administration of substance P and CGRP was due to a large extent to increased duration of the response, suggesting that the metabolism of the neuropeptides was altered. The presence of membrane-bound peptidases is thought to be the main mechanism determining the intensity and duration of the response to activation of primary afferent nerves by neuropeptides. Substance P and CGRP are both metabolized by NEP.11,12 However, the kinetics for the peptide-enzyme interaction are quite different for the 2 neuropeptides. CGRP is cleaved much less rapidly than substance P,11 suggesting that inhibition of NEP would have a greater effect on the responses to substance P than CGRP. Enzymatic degradation of one neuropeptide is retarded in the presence of another neuropeptide that is broken down by the same enzyme, as demonstrated by the fact that CGRP inhibits the metabolism of substance P.12 Studies showing that CGRP produced a similar increased intraspinal spreading of substance P in response to peripheral stimulation as did inhibitors of NEP provide further evidence that CGRP retards the metabolism of substance P.17 We tested the hypothesis that the synergism between CGRP and substance P in the activation of renal sensory neurons was related to CGRP binding to and occupying NEP, thus retarding the metabolism of substance P. We compared the ARNA responses to those when the neuropeptides were given alone and together, before and during inhibition of NEP with thiorphan. We reasoned that if the enhanced ARNA response to the combined administration of substance P and CGRP was due mainly to CGRP preventing the metabolism of substance P, then inhibiting NEP with thiorphan would eliminate the CGRP-mediated enhancement of the effects of substance P. Therefore, thiorphan would enhance the ARNA response to substance P but not to CGRP, and the ARNA response to substance P+CGRP would be similar to that produced by substance P alone. However, if CGRP enhanced the ARNA response to substance P by a mechanism that was not related to binding to NEP, thiorphan would enhance the ARNA responses to both substance P and CGRP, and the ARNA response to the combined administration of the 2 neuropeptides would be larger than in the absence of thiorphan. The data show that in the presence of thiorphan, the ARNA response to substance P+CGRP was similar to that produced by substance P alone. Whereas the ARNA response to substance P alone tended to be larger in the presence than absence of thiorphan, the ARNA response to CGRP tended to be smaller in the presence of thiorphan. The lack of statistical significance between the ARNA responses to substance P and CGRP in the absence and presence of thiorphan was most likely related to the experimental design. During the course of the experiment, 105 minutes elapsed between the 2 administrations of substance P, CGRP, and substance P+CGRP. Individual data show that the ARNA response to substance P was doubled in the presence of thiorphan in 6 of 10 rats, and the ARNA response to CGRP was reduced by more than half in the presence of thiorphan in 7 of 10 rats. These results argue for the hypothesis that CGRP serves as a modulator in the activation of renal sensory nerves: CGRP increases the availability of substance P by retarding its metabolism by competing for the same catabolic enzyme, NEP.

CGRP: Activation of Substance P Receptors

Our studies with the NEP inhibitor suggested that CGRP may increase ARNA at least in part by activating substance P receptors. However, it remained unclear whether the ARNA response to CGRP still present during NEP inhibition was related to activation of renal sensory nerves by CGRP per se or a CGRP-mediated increase in substance P levels. Previous studies examining the interaction between substance P and CGRP in central and peripheral afferent nerves would support the hypothesis that the increase in ARNA by CGRP would be due to activation of substance P receptors.18,19 A substance P receptor antagonist completely blocked the inhibition of excitatory transmission in parabrachial nucleus caused by either CGRP or substance P.18 Likewise, the CGRP-mediated inhibition of gastric motility was blocked by a substance P receptor antagonist.19 In the present study, renal pelvis was perfused with the substance P receptor antagonist RP67580,15 which has high affinity for rat substance P receptors. RP67580 was administered at a concentration that we have previously shown blocks the ARNA response to substance P.1 The ARNA response to CGRP was almost completely blocked by RP67580. The inactive racemic enantiomer RP68651 had no effect. These findings are consistent with the hypothesis that CGRP stimulates renal pelvic sensory nerves by activating renal pelvic substance P receptors.

In summary, the results of the present study show a synergism between substance P and CGRP in the activation of renal pelvic sensory nerves, mainly due to a prolongation of the response. The enhanced ARNA response to the combined administration of substance P+CGRP compared with that produced by either neuropeptide alone was prevented by NEP inhibition. Furthermore, our data show that the ARNA response to CGRP was blocked by a substance P receptor antagonist. Taken together, our findings suggest that CGRP activates renal pelvic sensory nerves by retarding the metabolism of substance P, thereby increasing the amount of substance P available for stimulation of substance P receptors. These studies are consistent with the hypothesis that CGRP serves as a neuromodulator in the activation of renal pelvic sensory nerves.

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