Renal Sensory Receptor Activation by Calcitonin Gene–Related Peptide
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Abstract  In anesthetized rats we examined whether calcitonin gene–related peptide activated renal pelvic sensory receptors and, if so, whether activation of renal pelvic calcitonin gene–related peptide receptors contributes to the inhibitory renal reflex response to renal mechanoreceptor stimulation. Calcitonin gene–related peptide (0.0026, 0.026, 0.26, and 2.6 μmol/L) administered into the renal pelvis increased ipsilateral afferent renal nerve activity in a concentration–dependent fashion (32 ± 14%, 69 ± 19%, 93 ± 26%, and 253 ± 48% [all P < .01], respectively). The increases in ipsilateral afferent renal nerve activity elicited by calcitonin gene–related peptide were associated with increases in contralateral urinary sodium excretion. The calcitonin gene–related peptide receptor antagonist human CGRP (h-CGRP) (8-37) (0.01, 0.1, 1.0, and 10 μmol/L) decreased the ipsilateral afferent renal nerve activity response to renal pelvic administration of calcitonin gene–related peptide (0.26 μmol/L) in a concentration–dependent fashion (29 ± 4%, 33 ± 12%, 76 ± 9% [P < .01], and 86 ± 13% [P < .01], respectively). In the presence of renal pelvic perfusion with vehicle, an increase in ureteral pressure of 5, 10, and 20 mm Hg increased ipsilateral afferent renal nerve activity by 13 ± 7%, 41 ± 7% (P < .01), and 95 ± 15% (P < .01) and contralateral urinary sodium excretion by 8 ± 1%, 24 ± 4%, and 42 ± 7% (all P < .05). The ipsilateral afferent renal nerve activity and contralateral natriuretic responses to graded increases in ureteral pressure (5 to 20 mm Hg) were unaltered by renal pelvic perfusion with h-CGRP (8-37) at 1.0 and 10 μmol/L. The data suggest that there are sensory receptors in the renal pelvic area that are responsive to calcitonin gene–related peptide. Activation of these receptors elicits a contralateral natriuretic response similar to that produced by renal mechanoreceptor stimulation. However, activation of renal calcitonin gene–related peptide receptors does not contribute to renal mechanoreceptor activation. (Hypertension. 1994;23[part 2]:1063–1067.)

Key Words • mechanoreceptors • afferent renal nerve activity • kidney

Calcitonin gene–related peptide (CGRP) is a 37–amino-acid peptide formed as the result of the alternative processing of transcription of the calcitonin gene.1 CGRP has been localized in several areas of the central and peripheral nervous systems.2 In the kidney the majority of the CGRP-containing sensory neurons have been localized to the renal pelvis,3 where sensory neurons have been shown to contain substance P in addition to CGRP.4 Based on the neuropeptide content, there appear to be at least four separate populations of sensory neurons present in the renal pelvis: two large groups containing either substance P or CGRP alone and two small groups containing either both peptides or neither.4 We have previously shown that activation of renal pelvic sensory receptors by substance P elicits a similar reflex increase in contralateral urinary sodium excretion as renal mechanoreceptor (MR) stimulation by increased ureteral pressure.5 The increases in ipsilateral afferent renal nerve activity (ARNA) and contralateral urinary sodium excretion produced by renal MR stimulation were blocked by the renal pelvic substance P receptor antagonist CP-96,345 but were unaffected by CP-96,344, the 2R,3R enantiomer of CP-96,345 that has much less affinity for substance P receptors.5 Furthermore, the renal reflex responses to renal MR stimulation were blocked by chronic pretreatment with capsaicin to deplete sensory neurons of substance P.5 Taken together these studies suggested that activation of renal pelvic substance P receptors contributes to renal MR stimulation.

The presence of CGRP-containing neurons in the renal pelvis1–4 and chronic treatment with capsaicin, depleting sensory neurons not only of substance P but also of CGRP,7 suggest that renal MR stimulation also involves activation of CGRP-containing neurons. Therefore the present study was performed to examine whether CGRP activates renal sensory receptors and, if so, whether activation of renal pelvic CGRP receptors contributes to renal MR stimulation.

Methods
Experiments were performed in male Sprague-Dawley rats (Harlan Sprague Dawley Inc) weighing 256 to 410 g (mean weight, 341 ± 9 g). 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. Anesthesia was induced with 50 mg/kg pentobarbital sodium IP (Nembutal, Abbott Laboratories) and maintained with an intravenous infusion of 10 mg·kg⁻¹·h⁻¹ in isotonic saline at 50 μL/min. Catheters were inserted into the femoral artery for measurement of arterial pressure (Statham transducer P23Db, Gould) and the femoral vein for pentobarbital sodium infusion. Heart rate was recorded by a linear cardiograph (Beckman 9857, Sensor Medics) triggered by the arterial pressure.
All recordings were made on a Beckman R-611 Dynograph recorder that was connected to an IBM PS/2 model 30 via a
Data Translation A/D board (model DT 2801) for on-line data acquisition.

A left flank incision was performed, and a polyethylene catheter (PE-10) was inserted into the right ureter for urine collection.

For administration of drugs into the left renal pelvis, a PE-10 catheter was inserted into a PE-60 catheter placed in the left ureter and advanced into the renal pelvis with its tip ending 1 to 2 mm beyond the tip of the PE-60 catheter.6,4 This technique allowed complete drainage of the perfusate. The renal pelvis was perfused with vehicle, the CGRP receptor antagonist human CGRP (h-CGRP) (8-37), or vehicle (0.15 mol/L NaCl) according to the protocols for groups 1 through 5 at a rate of 20 μL/min, a perfusion rate that did not increase ureteral pressure.6

For renal MR stimulation, ureteral pressure was increased 5, 10, and 20 mm Hg by elevating the 50-cm-long catheter (PE-60) inserted into the left ureter and filled with 0.15 mol/L NaCl.6,4 Ureteral pressure was recorded with a P23Db Statham transducer connected to the ureteral catheter by a T-tube connector.

For recording of renal nerve activity, one renal nerve branch was isolated between the angle of the aorta and the left renal artery with the use of a dissecting microscope. Recordings from multitherm preparations were made by placing the renal nerve on a bipolar silver-wire electrode (Cooner Wire) that was fixed to the renal nerve with silicone cement (Wacker Sil-Gel 604, Wacker-Chemie). The signals were led by a high-impedance probe (HIP511, Grass Instrument Co) to a band-pass amplifier (Grass P515) with a high- and low-frequency cutoff of 3000 and 30 Hz, respectively. The signals were amplified 20,000-fold. The output of the band-pass 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. Assessment of renal nerve activity was done by its pulse-synchronous rhythmicity. On establishment of optimal renal nerve activity, we sectioned the renal nerve and placed the distal part on the electrode for recording of ARNA. Background noise level was assessed by crushing the decentralized renal nerve bundle peripheral to the recording electrode. The integrated background noise level was subtracted from the integrated total signal in microvolts times second per 1-second interval. The background noise level after crushing the nerve was zero in 31 rats. In the remaining 11 rats, the signal-to-noise ratio averaged 4.3±0.5. After subtraction of the background noise level, we expressed ARNA in percent of its first control value.

Experimental Procedures

Approximately 1.5 hours elapsed between the end of surgery and the start of the experiment.

**Group 1: CGRP-Dose-Response Curve**

Four 5-minute control, experimental, and recovery periods were separated by 20-minute intervals. The renal pelvis was perfused throughout the experiment (n=12) with vehicle (0.15 mol/L NaCl) except during the first 2.5 minutes of each experimental period, when the renal perfusate was switched from vehicle to CGRP at 0.0026, 0.026, 0.26, or 2.6 μmol/L (random order); the total volume of administered CGRP was 50 μL.

**Group 2: CGRP–CGRP Receptor Antagonist**

Five 5-minute control, experimental, and recovery periods were separated by 20-minute intervals. The renal pelvis was perfused with vehicle (0.15 mol/L NaCl) during the first control and recovery periods and the CGRP receptor antagonist h-CGRP (8-37)6 (0.001, 0.1, 1.0, and 10 μmol/L) during the next four control and recovery periods. The renal pelvic perfusate was switched to the next higher concentration of h-CGRP (8-37) immediately after each recovery period. During the first 2.5 minutes of each experimental period, the renal pelvic perfusate was switched from vehicle or h-CGRP (8-37) to 0.26 μmol/min CGRP.

**Groups 3 Through 5:**

**Renal MR Stimulation–h-CGRP (8-37)/Vehicle**

In group 3 (n=8), three 10-minute control, 5-minute experimental, and 10-minute recovery periods separated by 20-minute intervals were performed twice. Durin the experimental periods, ureteral pressure was increased 5, 10, and 20 mm Hg (random order). At the end of the third recovery period of each part of the experiment, 0.26 μmol/L CGRP was administered into the renal pelvis for 2.5 minutes. The renal pelvis was perfused with vehicle (0.15 mol/L NaCl) throughout the experiment.

In group 4 (n=9), the experimental protocol was similar to that in group 3 except the second control, experimental, and recovery periods were performed in the presence of renal pelvic perfusion with 1 μmol/L h-CGRP (8-37). The renal pelvic perfusate was switched from vehicle to h-CGRP (8-37) immediately after the third recovery period.

In group 5 (n=4), the experimental protocol was similar to that in group 4 except the renal pelvis was perfused with 10 μmol/L h-CGRP (8-37) during the second portion of the experiment.

**Chemical Analyses**

Urinary Na+ concentrations were determined with a flame photometer (model 143, Instrumentation Laboratories). Right (contralateral) urinary sodium excretion was expressed per gram of kidney weight.

**Drugs**

CGRP and h-CGRP (8-37) (Sigma Chemical Co) were dissolved in 0.15 mol/L NaCl.

**Statistical Analysis**

Left (ipsilateral) ARNA, systemic hemodynamics, and right (contralateral) renal excretion were measured and averaged over each period. Peak values of ipsilateral ARNA were measured during administration of CGRP. The effects of CGRP and renal MR stimulation were evaluated by comparing the value observed during the experimental period with the average of the bracketing control and recovery values. Friedman's two-way analysis of variance, shortcut analysis of variance, Wilcoxon's matched-pairs signed rank test, and the Mann-Whitney U test were used. A significance level of 5% was chosen. Data are expressed as mean±SEM.

**Results**

**Group 1: CGRP–Dose-Response Curve**

The results are shown in Fig 1. Renal pelvic administration of CGRP (0.0026 to 2.6 μmol/L) increased ipsilateral ARNA in a concentration-dependent fashion. The duration of the response to CGRP increased with increasing concentration, from 11±4 seconds (0.026 μmol/L) to 49±7 seconds (2.6 μmol/L). Contra-lateral urinary sodium excretion increased by 21±5% from 1.3±0.2 μmol/min • g−1 and by 22±9% from 1.5±0.2 μmol • min−1 • g−1 (both P<.01) by 0.26 and 2.6 μmol/L of CGRP, respectively. Mean arterial pressure (119±5 mm Hg) and heart rate (315±11 beats per minute) were unaffected by CGRP.

**Group 2: CGRP–CGRP Receptor Antagonist**

The results are shown in Fig 2. Before administration of h-CGRP (8-37), renal pelvic administration of CGRP (0.26 μmol/L) resulted in a similar increase in ipsilateral ARNA and contralateral urinary sodium excretion...
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(22±8%; P<.02) as in group 1. Renal pelvic perfusion with h-CGRP (8-37) (0.01 to 10.0 μmol/L) did not affect baseline mean arterial pressure (108±5 mm Hg), heart rate (334±8 beats per minute), or contralateral urinary sodium excretion (1.6±0.2 μmol • min⁻¹ • g⁻¹). However, h-CGRP (8-37) decreased the ipsilateral ARNA response to 0.26 μmol/L CGRP in a concentration-dependent fashion; maximal blockade was produced by 1 μmol/L h-CGRP (8-37). The contralateral natriuretic responses to CGRP were reduced by 1 and 10 μmol/L h-CGRP (8-37).

**Group 3: Renal MR Stimulation—Vehicle**

Increasing ureteral pressure 5, 10, and 20 mm Hg resulted in graded increases in ipsilateral ARNA (13±7%, 41±7% [P<.01], and 95±15% [P<.01], respectively) and in contralateral urinary sodium excretion (8±4%, 24±4%, and 42±7% [all P<.05], respectively). Repeated increases in ureteral pressure of the same magnitude resulted in reproducible increases in ipsilateral ARNA (23±14%, 45±6% [P<.01], and 78±19% [P<.01], respectively) and contralateral urinary sodium excretion (8±2%, 20±4%, and 40±4% [all P<.05]). Likewise, repeated administration of CGRP (0.26 μmol/L) resulted in reproducible responses (189±29% and 155±26%, P<.01). Although there was a small gradual decrease in basal mean arterial pressure during the course of the experiment, from 118±4 to 107±4 mm Hg, mean arterial pressure was unaffected by increased ureteral pressure.

**Groups 4 and 5: Renal MR Stimulation—h-CGRP**

The results are shown in Fig 3. In the presence of renal pelvic perfusion with 0.15 mol/L NaCl, the ipsilateral ARNA and the contralateral natriuretic responses to graded increases in ureteral pressure were similar to those in group 3. The ipsilateral ARNA and contralateral natriuretic responses to graded increases in ureteral pressure were unaltered by renal pelvic perfusion with h-CGRP at 1 μmol/L. Similarly, renal pelvic perfusion with h-CGRP (8-37) at 10 μmol/L failed to reduce the ipsilateral ARNA responses to graded increases in ureteral pressure. The increases in ipsilateral ARNA produced by graded increases in ureteral pressure were 16±3%, 48±23%, and 98±30% during renal pelvic perfusion with vehicle and 45±14%, 63±26%, and 176±58% during renal pelvic perfusion with 10 μmol/L h-CGRP (8-37). However, the ipsilateral ARNA responses to 0.26 μmol/L CGRP were blocked by 83±6% and 72±15% by 1 and 10 μmol/L h-CGRP, respectively. In group 4, there was a similar gradual decrease in basal mean arterial pressure (from 125±6 to 119±7 mm Hg) as in group 3. Basal mean arterial pressure (116±6 mm Hg) remained unaltered throughout the experiment in group 5.

**Discussion**

The present study demonstrates that renal pelvic administration of CGRP increases ipsilateral ARNA and contralateral urinary sodium excretion in the absence of changes in arterial pressure. The increases in ipsilateral ARNA and contralateral urinary sodium excretion elicited by CGRP were blocked by renal pelvic...
perfusion with the CGRP-selective receptor antagonist h-CGRP (8-37), suggesting that CGRP activates renal sensory receptors located in the renal pelvic area. Renal pelvic perfusion with h-CGRP (8-37) did not alter the ipsilateral ARNA responses to increased ureteral pressure. These results suggest that activation of renal pelvic CGRP receptors does not contribute to renal MR activation.

There is substantial evidence of widespread presence of CGRP in both central and peripheral sensory neurons. Although there is considerable evidence for a role of CGRP in depolarization of neurons in spinal dorsal horn, there is little information on the effects of CGRP on peripheral primary afferent nerves. In vitro studies by Palmer et al. showed an excitatory effect of CGRP on myenteric neurons of the guinea pig ileum. An in vivo study in rabbits that compared the baroreflex responses to intravenous CGRP and nitroprusside suggested that the enhanced baroreflex-mediated increase in efferent renal nerve activity produced by CGRP compared with equidepressor doses of nitroprusside was related to a central effect of CGRP. In the rat kidney immunohistochemical studies have localized the majority of CGRP as well as substance P-containing neurons to the pelvic area. Functional support for renal pelvic sensory neurons containing CGRP and substance P derives from studies using capsaicin, an agent known to cause a release of CGRP and substance P from sensory neurons at low concentrations. Superfusion of an isolated renal pelvic preparation with capsaicin increased the release of CGRP and substance P. Furthermore, our previous studies have shown that capsaicin caused a greater ipsilateral ARNA response when injected into the renal pelvis versus the renal interstitium. Because these studies suggested the presence of CGRP receptors in the renal pelvic area, CGRP was administered into the renal pelvis at increasing concentrations in the present study. Our findings showed that renal pelvic administration of CGRP at 0.0026 to 2.6 μmol/L increased ipsilateral ARNA in a concentration-dependent fashion in the absence of changes in arterial pressure and heart rate. The duration of the ARNA response to 0.26 μmol/L CGRP averaged 43±4 seconds (n=41). Renal pelvic sensory receptors were activated by renal pelvic administration of CGRP at concentrations (≥2.6 nmol/L) shown to inhibit renal pelvic contraction, relax afferent renal arterioles, and increase adenylate cyclase activity in rat membrane preparations from glomeruli and renal medulla and papilla. Although the location of the renal pelvic sensory receptors is not known, it is most likely that the concentration of CGRP reaching the sensory receptors was even less than that of the perfusate. Also the CGRP concentration would be diluted by the continuous urine production. The increases in ipsilateral ARNA produced by CGRP at 0.26 and 2.6 μmol/L were associated with an increase in contralateral urinary sodium excretion, i.e., a response similar to that produced by renal pelvic administration of substance P. The increases in ipsilateral ARNA and contralateral urinary sodium excretion to CGRP at 0.26 μmol/L (submaximal concentration) were blocked by renal pelvic perfusion with the CGRP-selective receptor antagonist h-CGRP (8-37) in a concentration-dependent fashion. Maximal blockade of the ARNA response to CGRP was produced by h-CGRP (8-37) at 1 μmol/L. The C-terminal fragment of h-CGRP (8-37) is a specific antagonist for CGRP receptors. h-CGRP (8-37) produces a selective inhibition of various CGRP-mediated effects in rats, including increases in plasma membrane and intracerebral arterioles, vasodilation in various vascular beds, and renal pelvic relaxation. The findings of these studies, together with those of the present study suggest that CGRP increased ipsilateral ARNA through activation of renal pelvic CGRP receptors.

In agreement with our previous studies, graded increases in ureteral pressure resulted in increased ARNA and contralateral urinary sodium excretion. The renaloren reflex responses to renal MR stimulation are impaired in rats receiving long-term treatment with high doses of capsaicin. Because chronic capsaicin treatment causes depletion of both CGRP and substance P from sensory neurons and decreases CGRP levels in renal medulla and papilla, these studies suggested that activation of substance P receptors and/or CGRP-containing neurons contributes to renal MR stimulation. Our previous studies suggested that activation of the ipsilateral ARNA response to renal MR stimulation by the sodium channel blocker lidocaine suggested that these receptors were functionally localized to the renal pelvis. However, renal pelvic perfusion with the CGRP receptor antagonist h-CGRP (8-37) failed to alter the ARNA responses to graded increases in ureteral pressure at concentrations (1 and 10 μmol/L) shown to block the ARNA response to CGRP. Although in the present study renal nerve activity was recorded from a multifiber renal nerve preparation, it is most likely that the increase in ARNA produced by increased ureteral pressure was related to activation of renal MR, because the magnitude of the ARNA response was related to the magnitude of the increase in ureteral pressure with the renal pelvis being perfused with the same perfusate. Taken together these findings suggest that activation of renal pelvic CGRP receptors does not contribute to renal MR activation. The lack of an effect of a CGRP receptor antagonist on the renorenal reflex responses to renal MR stimulation is in contrast to our previous findings showing that the renorenal reflex responses to renal MR stimulation were blocked by the substance P receptor antagonist CP-96,345; CP-96,344, the 2R,3R enantiomer of CP-96,345 that has much less affinity for substance P receptors, was without effect. Taken together these studies suggest that activation of substance P receptors but not CGRP receptors contributes to the renorenal reflex responses to renal MR stimulation.

It has also been hypothesized that CGRP and substance P released from the afferent renal pelvic nerves may play a modulatory role in renal pelvic motility. Administration of CGRP was found to inhibit and substance P was found to increase spontaneous renal pelvic contractions of isolated renal pelvic muscle strips. Because ARNA is increased by both CGRP and substance P, it may be argued that the increase in ARNA produced by CGRP is not related to CGRP-mediated relaxation in the present study, unless renal pelvic sensory receptors are activated by both decreased and increased renal pelvic smooth muscle activity. There is considerable evidence for both CGRP and substance P being potent vasodilators in various vascu-
lar beds. Whether renal pelvic administration of CGRP altered intrarenal hemodynamics was not examined in the present study. However, our previous studies showed that renal pelvic administration of substance P increased ARNA in the absence of any changes in renal interstitial hydrostatic pressure.

The physiological significance of renal pelvic CGRP receptors is unknown. In addition to MR, two classes of chemoreceptors, R1 and R2, have been identified in the renal pelvic area. Both R1 and R2 are activated by renal ischemia. R2 is also activated by renal pelvic perfusion with 0.05 to 0.15 mol/L KCl, >0.5 mol/L NaCl, and 1 mol/L mannitol. In vitro studies in urinary bladder have shown that CGRP can be released by hypertonic NaCl, high K+ concentration, and protons. Therefore one might postulate a possible role for CGRP in renal chemoreceptor activation.

In summary, the results of the present study demonstrate the presence of CGRP receptors in the renal pelvic area. Activation of these receptors results in an increase in contralateral urinary sodium excretion, i.e., a response analogous to that produced by renal MR stimulation. However, the renorenal reflex responses to renal MR stimulation were unaltered by a CGRP receptor antagonist, suggesting that activation of renal CGRP receptors does not contribute to renal MR activation.

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