Atrial Natriuretic Peptides Increase Calcitonin Gene–Related Peptide Within Human Circulation

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Long-acting natriuretic peptide (LANP), vessel dilator, and atrial natriuretic factor (ANF) (each infused at 100 ng/kg body weight [BW] · min for 60 minutes) increased the circulating concentration of calcitonin gene–related peptide (CGRP) threefold to fourfold in 30 healthy humans. Within 30 minutes of stopping ANF infusion, the CGRP circulating concentration had returned to preinfusion levels, whereas its increase secondary to the other atrial peptides was still significant 2 to 3 hours after cessation of their infusions. There was a 50% decreased excretion ($P < .001$) of CGRP into the urine secondary to LANP and vessel dilator, which correlated with the increase of CGRP in the circulation. The ANF-induced 50% decreased CGRP excretion occurred after the circulating concentration of CGRP had returned to preinfusion levels. Kaliuretic peptide did not affect CGRP circulating concentration or excretion into urine. These data suggest that LANP and vessel dilator inhibit the metabolic breakdown of CGRP as part of their mechanism of increasing CGRP in plasma, whereas the ANF effect of increasing CGRP in plasma appears to be secondary to stimulating the release of CGRP.

CALCITONIN GENE–RELATED PEPTIDE (CGRP) is a 37–amino acid (aa) peptide produced by alternative processing of the calcitonin gene.[1] CGRP is a potent vasodilator in both animals[2] and humans.[3] CGRP has natriuretic and diuretic effects that have been associated with its ability to increase atrial natriuretic factor (ANF).[4] CGRP increases ANF both in vitro[5,6] and in vivo.[4]

The atrial natriuretic peptide hormone system consists of a 126-aa prohormone synthesized within myocytes of the heart and stored in storage granules within the heart for release into the circulation[7,8] (Fig 1). This hormonal system contains several peptides from the same 126-aa prohormone with blood pressure–lowering, natriuretic, diuretic, and/or kaliuretic (ie, potassium-excreting) properties.[9-11] Thus, peptides consisting of aa 1 to 30 (long-acting natriuretic peptide [LANP]), aa 31 to 67 (vessel dilator), aa 79 to 98 (kaliuretic peptide), and aa 99 to 126 (ANF) each have blood pressure–lowering, diuretic, natriuretic, and/or kaliuretic properties in both humans[10,11] and animals.[9,12] When released into the circulation, these peptides circulate as a 98-aa *N*-terminus and a 28-aa C-terminus (ie, ANF) of this prohormone.[13-15] In addition, vessel dilator, LANP, and kaliuretic peptide circulate as distinct entities after being proteolytically cleaved from the rest of the *N*-terminus by protease(s)[11,16,17] (Fig 1). Both the *N*- and C-terminal ANF prohormone peptides are released simultaneously with central hypervolemia[17,18] and with rapid heart rates of 125 bpm or greater.[19,20] These peptides are also released simultaneously in vitro from isolated perfused rat atria by atrial distension.[21]

Each of the atrial natriuretic peptides have a negative feedback on the release of each other.[10] Thus, infusion of ANF decreases the release of LANP, vessel dilator, and kaliuretic peptide.[10] Likewise, each of the other atrial peptides derived from the ANF prohormone decrease the release of ANF and of each other.[10] The present investigation was designed to determine if LANP, vessel dilator, kaliuretic peptide, and/or ANF influence the release and/or metabolism of CGRP in humans. Since CGRP has biologic effects similar to each of these atrial peptides[2-4] and may mediate some of their effects, we tested the ability of LANP, vessel dilator, kaliuretic peptide, and ANF to influence the release and/or metabolic breakdown of CGRP by measuring its circulating level and urinary excretion before, during, and for 3 hours after infusion of each of the respective atrial peptides at 100 ng/kg · body weight (BW) · min for 60 minutes.

SUBJECTS AND METHODS

Subjects

Thirty healthy subjects (15 men and 15 women aged 22 to 58 years; mean, 32 years; all normotensive with blood pressure <125/80 mm Hg) were studied. They had heart rates ranging from 56 to 80 bpm, with respiration rates between 12 and 14 per minute. These volunteers were divided into five similar groups based on age, sex, weight, blood pressure, and heart rate. The characteristics of each individual in this investigation have been published[11] in an investigation of potassium and sodium excretion following administration of each of the atrial peptides. None of the volunteers had any known disease. Of importance, none of the subjects had any abnormality of sodium or water metabolism. None were taking any medication. Informed consent was obtained from each subject after the nature and possible consequences of the studies were fully explained. This investigation followed the recommendations of the Declaration of Helsinki on investigations with human subjects. The study was approved by the Institutional Review Board of the University of South Florida Health Sciences Center and the Research Committee of the James A. Haley Veterans Hospital. It was also approved by the US Food and Drug Administration (FDA IND No. 32,119).

Experimental Protocol

After obtaining written informed consent, an Insyte-w 20-gauge, 1.5-in catheter (Becton Dickinson Vascular Access, Sandy, UT) was placed into the forearm of each subject for infusion and blood sampling. A 60-minute baseline period preceded any infusion. A total volume of 20 mL normal saline (0.9% sodium chloride) with or without atrial natriuretic peptides was infused by a constant-rate infusion pump over a

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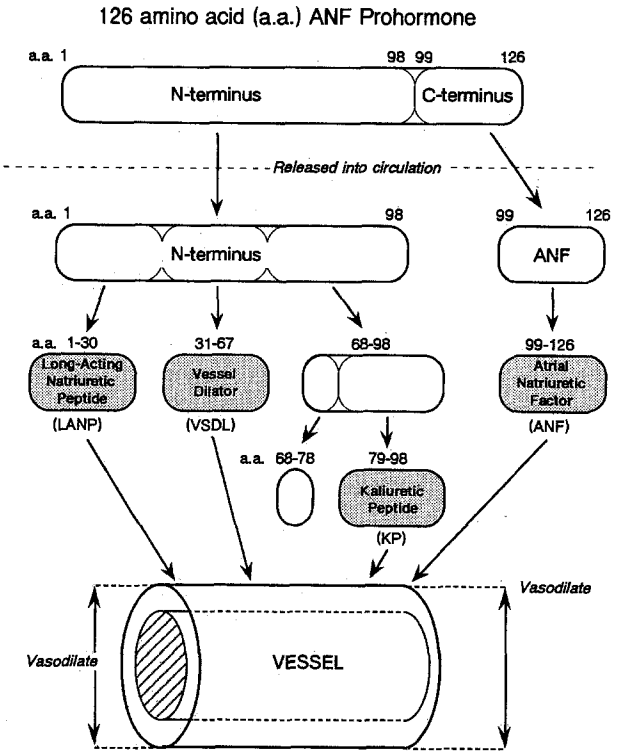

Fig 1. Origination of LANP, vessel dilator, kaliuretic peptide, and ANF from the 126-aa ANF prohormone. LANP consisting of aa 1 to 30, vessel dilator (aa 31 to 67), and kaliuretic peptide (aa 79 to 98) originate from the *N*-terminus, whereas ANF (aa 99 to 126) originates from the C-terminus of this prohormone. Each of these peptide hormones circulates as a distinct entity and each decreases blood pressure.

60-minute period. Blood samples were obtained every 20 minutes during the infusion and at 30-minute intervals during the 1-hour baseline and 3-hour postinfusion periods. Thus, one group (control group, or sham infusion) received only 20 mL normal saline without atrial natriuretic peptides, and the other groups received the respective atrial peptides in 20 mL normal saline. All subjects were studied in the morning after an overnight fast, beginning a baseline period at 8 AM. Each volunteer was studied in the seated position and received only one peptide infusion. Blood pressure was monitored every 5 minutes throughout the investigation. One hundred nanograms per kilogram BW per minute was chosen for the infusion dosage of these atrial natriuretic peptides because the rate of release of *N*-terminal ANF prohormone peptides from the atrium of the heart with physiological stimuli is 138 to 292 ng/kg BW · min, whereas the release rate of ANF from the atrium is 76 ng/kg BW · min.[22] Molar equivalents of the 100-ng/kg BW dose are 32, 29, 26, and 46 pmol/kg BW for ANF, LANP, vessel dilator, and kaliuretic peptide, respectively. After completion of the 60-minute baseline period, to maintain a similar plasma volume throughout the study, orange juice (Na^+, 0.001 mmol/L; K^+, 0.046 mmol/L) was given orally in milliliters for each milliliter of urine output at the above times. Each volunteer received only one peptide infusion.

Purity of Atrial Natriuretic Peptides

The human forms of the atrial natriuretic peptides (LANP, vessel dilator, kaliuretic peptide, and ANF) were synthesized by Peninsula Laboratories (Belmont, CA). Before use in these studies, samples of these commercially synthesized peptides were subjected to high-performance liquid chromatography (HPLC) to determine purity using a Novapak C_{18} (5 μm) cartridge column (Waters Chromatography Division, Millipore, Milford, MA). The flow rate for the HPLC study was 1 mL/min with 0.1% trifluoroacetate solvent in pump A and 60% acetonitrile in 0.1% trifluoroacetate in pump B, with a gradient of 0% to 60% acetonitrile achieved in 40 minutes. This evaluation verified the purity and authenticity of atrial natriuretic peptides compared with their known HPLC elution profile.[15] After determining that the atrial natriuretic peptides were pure, they were then dissolved in 0.9% saline solution in the hospital pharmacy, where pyrogen and sterility testing were performed before dispensing the 100-ng/kg BW concentrations into two 10-mL syringes. The entire contents of each 10-mL syringe were infused over a 30-minute period. After completing the experiment, each of the syringes and the infusion catheter were examined by radioimmunoassays (RIAs) to determine the amount of the atrial natriuretic peptide that may have remained within the syringes or tubing. Approximately 5% of atrial natriuretic peptides that were infused remained on the walls of the syringes and tubing after completion of the infusion. This was determined after completion of the experiment by flushing the syringes three times with 4 mL 0.9% saline and then measuring, by atrial natriuretic peptide RIAs, how much of the respective atrial peptides were present in the 0.9% saline flushes. The amount measured was then compared with the amount infused to determine the percentage remaining on the walls of the syringes and tubing.

Measurement of Atrial Natriuretic Peptides and CGRP

Each of the blood samples and the results of flushing the syringes and tubing with 4 mL 0.9% sodium chloride were collected into chilled 5-mL EDTA tubes to prevent proteolytic breakdown of any peptides that might be present. These samples were transported on ice and immediately centrifuged at 3,000 × *g* for 15 minutes. After centrifugation, each sample was extracted with 100% ethanol (1:2 dilution), vortexed, and allowed to stand at 4°C for 30 minutes.[15] Atrial natriuretic peptide levels were measured by RIAs described previously by our laboratory.[11,15,23] The extracted plasma was first reconstituted in 100 μL 0.1-mol/L phosphate buffer (pH 7.4) containing 0.05 mol/L NaCl, 0.1% bovine serum albumin, 0.1% Triton X-100, and 0.01% NaN_3. To the redissolved sample, 100 μL (0.03 mg) rabbit immunoglobulin G plus 100 μL ^{125}I-labeled atrial natriuretic peptides (10,000 cpm) were added, mixed, and incubated for 18 hours at 4°C. Precipitation of the antibody-bound tracer was accomplished by adding 100 μL goat antirabbit globulin after the above-described 18-hour period and incubating this mixture for 2 hours at room temperature. Each tube was then centrifuged at 3,000 × *g* for 20 minutes. The supernatant was aspirated, and the pellet was counted in a gamma counter. All determinations were performed in triplicate. Intraassay coefficients of variation for LANP, vessel dilator, kaliuretic peptide, and ANF RIAs were 4.8%, 5.3%, 5.5%, and 5.7%, respectively. Interassay coefficients of variation were 8% for LANP and vessel dilator, and kaliuretic peptide and ANF interassay variations were 7.6% and 6.9%. Recovery was examined by adding synthetic unlabeled LANP, vessel dilator, kaliuretic peptide, and ANF at 100, 200, and 400 pg/mL to pooled plasma. Recovery for LANP was 83.5% ± 13.2%; vessel dilator recovery was 100.9% ± 8.9%. Recovery of kaliuretic peptide was 86.8% ± 9.6% and of ANF 92% ± 11%. The respective IC_{50}s were 180, 120, 47, and 11 fmol/tube; the lowest detectable concentrations were 40, 35, 5, and 1.4 fmol/tube for LANP, vessel dilator, kaliuretic peptide, and ANF RIAs, respectively. Serial dilution of pooled plasma has demonstrated excellent parallelism of standards and unknowns in these atrial natriuretic peptide assays.[15,18] There is not any cross-reactivity of CGRP in the ANF, LANP, vessel dilator, or kaliuretic peptide RIAs.

CGRP level was measured at each of the same time points as the atrial natriuretic peptides (ie, 0, 30, 60, 80, 100, 120, 150, 180, 210, 240, and 300 minutes) with a human CGRP RIA kit (Phoenix Pharmaceuticals, Belmont, CA). Before CGRP RIA, the blood samples collected into

EDTA tubes at the above time points were immediately centrifuged at 3,000 × *g* for 15 minutes and then extracted with 100% ethanol (1:2 dilution), vortexed, and allowed to stand at 4°C for 30 minutes. The extracted plasma was reconstituted in 100 μL Phoenix Pharmaceuticals buffer (pH 7.4) containing 0.1% Triton X-100, 0.1% bovine serum albumin, 0.05 mol/L NaCl, and 0.01% NaN_3. Each of the redissolved samples then had 100 μL antirabbit CGRP added, mixed, and incubated at 4°C for 18 hours. Subsequently, 100 μL ^{125}I-labeled CGRP was added and the samples were vortexed and incubated at 4°C for 18 hours. Precipitation of antibody-bound tracer was accomplished by adding 100 μL goat antirabbit immunoglobulin G serum and 100 μL normal rabbit serum after the above 18-hour period and incubating this mixture for 90 minutes at room temperature. Each sample was then centrifuged at 3,000 × *g* for 20 minutes. The supernatant was aspirated, and the pellet was counted in a gamma counter. The interassay coefficient of variation for the CGRP RIA was 8.3%, and the intraassay coefficient of variation was 6.4%. The IC_{50} of the CGRP RIA was 9 pmol/L (10 pg/tube). All determinations of CGRP were performed in duplicate. There is not any cross-reactivity of any of the atrial natriuretic peptides in the CGRP RIA. There is no cross-reactivity with calcitonin (31% structural homology with CGRP), amylin (37% homology with CGRP), adrenomedullin (20% homology with CGRP), or substance P in this CGRP RIA.

Statistical Analysis

The data obtained in this investigation are presented as the mean ± SD. Measurements obtained in the same subject over time were evaluated by repeated-measures ANOVA. Duncan's multiple range test (MRT) was used after ANOVA to evaluate which means were significantly different from baseline and from each other. Maximal changes in systolic and diastolic blood pressure within groups were determined by a paired Student's *t* test. Correlation coefficients determined between the respective atrial peptides and the increase in CGRP in plasma and its decrease in urine were made by the Spearman rank test. To be statistically significant, we required a *P* value of less than .05 (95% confidence limit).

RESULTS

Atrial Peptides Decrease Systolic and Diastolic Blood Pressure

LANP, vessel dilator, kaliuretic peptide, and ANF each decreased systolic and diastolic blood pressure (Fig 2). Each of these peptides decreased systolic blood pressure to a greater extent than diastolic blood pressure when infused at 100 ng · kg BW · min for 60 minutes (Fig 2). Vessel dilator decreased systolic (10-mm Hg decrease) and diastolic (6.3-mm Hg decrease) blood pressure more than the other peptides, but the amount of decrease in blood pressure secondary to vessel dilator was not significantly greater than with the other peptides. The control group, which received 20 mL 0.9% saline over 60 minutes, had no decrease in either systolic or diastolic pressure; rather, in both groups blood pressure increased slightly during the 4-hour combined infusion and postinfusion periods (Fig 2). In this and the results that follow, it should be pointed out that each individual who received an infusion of one of the respective peptides also serves as his or her own control. The 60-minute period in the figures (which is the period immediately before beginning one of the respective infusions) serves as the control (baseline) value in the individual subjects with which one can compare any effects observed at later time points in this investigation. The blood pressure decreases already

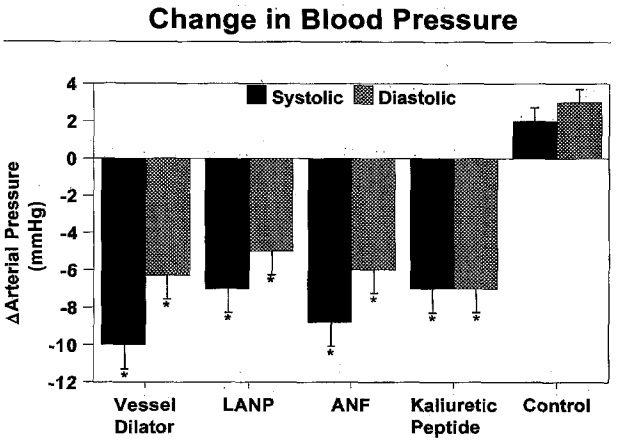

Fig 2. LANP, vessel dilator, ANF, and kaliuretic peptide decrease systolic and diastolic blood pressure in healthy human subjects. Decreases in both systolic and diastolic blood pressure secondary to LANP, vessel dilator, ANF, and kaliuretic peptide were significant at $P < .05$ when evaluated by paired *t* test. Values are the mean ± SEM of 6 healthy subjects.

referred to and the increase of CGRP in the circulation and/or decrease in the urine are the amount of decrease in blood pressure or change in the measured concentration of CGRP compared with the respective preinfusion measurement (ie, the 60-minute period) in each of the subjects.

LANP, Vessel Dilator, and ANF Increase CGRP in the Circulation

Infusion of ANF increased the CGRP circulating concentration fourfold ($P < .01$) within 20 minutes of starting ANF infusion, and CGRP remained increased by threefold (compared to the preinfusion concentration) throughout the 60-minute ANF infusion. Within 30 minutes of stopping ANF infusion, CGRP returned to its preinfusion concentration within the circulation. LANP also increased ($P < .01$) the CGRP circulating concentration (ie, threefold increase) within 20 minutes of starting infusion and remained increased by threefold throughout and for 30 minutes after stopping LANP infusion. CGRP was still increased by twofold ($P < .05$) within the circulation 2 hours after cessation of LANP infusion. The circulating concentration of CGRP did not begin to approach the preinfusion concentration until 3 hours after stopping LANP infusion (Fig 3).

Vessel dilator increased the circulating concentration of CGRP 3-fold within 20 minutes of starting its infusion. CGRP remained increased by threefold for 30 minutes after stopping the vessel dilator infusion. For 1 and 2 hours after stopping the vessel dilator infusion, the circulating concentration of CGRP was increased by twofold ($P < .05$) compared with the preinfusion and control concentrations. Three hours after stopping the vessel dilator infusion, the CGRP circulating concentration had returned to the preinfusion concentration. Kaliuretic peptide, on the other hand, did not affect the circulating concentration of CGRP (Fig 3).

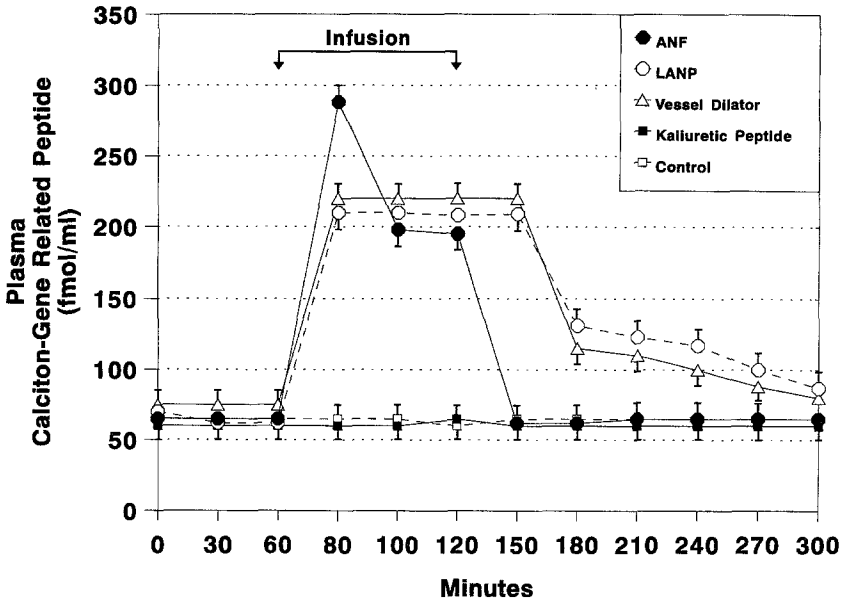

Fig 3. LANP, vessel dilator, and ANF increase circulating concentrations of CGRP in healthy humans, whereas kaliuretic peptide has no effect on CGRP. Controls received vehicle only. The increase in CGRP secondary to LANP, vessel dilator, and ANF was significant at $P < .01$ when evaluated by repeated-measures ANOVA followed by Duncan's MRT. Means ± SEM for CGRP at each time point are shown; n = 6 healthy subjects for each group.

LANP, Vessel Dilator, and ANF Decrease the Concentration of CGRP Excreted Into Urine

Infusion of LANP, vessel dilator, and ANF caused a rapid and significant ($P < .001$) decrease in the concentration of CGRP in the urine (Fig 4). Kaliuretic peptide, on the other hand, did not decrease the urinary concentration of CGRP. Twenty minutes after beginning the respective infusions, the concentration of CGRP had decreased within the urine by 30%, 47%, 0%, and 17% secondary to LANP, vessel dilator, kaliuretic peptide, and ANF, respectively. At the end of the respective atrial peptide infusions (ie, at 120 minutes), the concentration of CGRP had decreased to one third or less of the baseline amount present during the 60-minute preinfusion period for each of the peptides except kaliuretic peptide ($P < .001$). Three hours after stopping LANP and vessel dilator infusions, the concentration of CGRP had decreased further and was 20% or less than the original baseline concentration 3 hours postinfusion (Fig 4).

The concentration of CGRP within the urine after cessation of ANF infusion was distinctly different from the pattern observed with the other atrial peptides. After cessation of ANF infusion, the maximal decrease in the concentration of CGRP in the urine was 30 minutes postinfusion. One hour later (ie, 90 minutes after stopping ANF infusion), the concentration of CGRP had increased 2.5-fold. Three hours after ANF infusion, the concentration of CGRP had increased threefold from the lowest concentration in the urine at 30 minutes postinfusion and was nearly equal to the preinfusion value (Fig 4).

The excretion rate of CGRP (in femtomoles per milliliter urine per minute) secondary to infusion of each of the atrial peptides calculated at each time point showed that the maximal

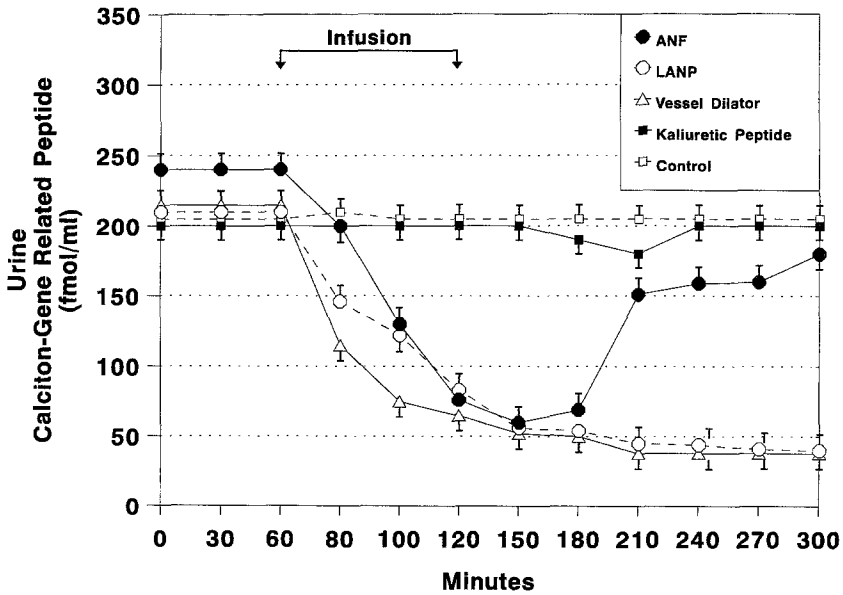

Fig 4. Vessel dilator, LANP, and ANF decrease concentrations of CGRP within the urine of healthy humans. The decrease in CGRP within the urine secondary to LANP, vessel dilator, and ANF was significant ($P < .05$) when evaluated by repeated-measures ANOVA followed by Duncan's MRT. Kaliuretic peptide did not decrease the urinary concentration of CGRP. Values are means ± SEM of 6 healthy subjects in each group.

decrease in excretion of CGRP was 47%, 50%, 5%, and 49% secondary to LANP, vessel dilator, kaliuretic peptide, and ANF, respectively ($P < .01$ for LANP, vessel dilator, and ANF; Table 1). In this table, one observes that the excretion rate of CGRP into the urine decreased within 20 minutes of starting the infusions of LANP and vessel dilator, and that the decrease in the rate of excretion into urine secondary to these atrial peptides was still significant ($P < .01$) 3 hours after stopping the infusions. The excretion rate of CGRP into the urine secondary to ANF, on the other hand, did not significantly decrease until 30 minutes after stopping the infusion (ie, it did not decrease during ANF infusion) and was maximal 1 hour after cessation of ANF infusion. The excretion of CGRP then returned to the pre–ANF infusion values 90 minutes after stopping ANF infusion. The inhibitory effects of LANP and vessel dilator on the excretion of CGRP into urine thus last significantly ($P < .001$) longer than those of ANF (Fig 4 and Table 1). The excretion rate of CGRP was not significantly affected by kaliuretic peptide (Table 1).

LANP, Vessel Dilator, and ANF Decrease the Renal Clearance of CGRP

During infusion of LANP, vessel dilator, and ANF, there was a significant ($P < .01$) 4.7-fold, 4.4-fold, and 3.9-fold, respectively, decrease in the renal clearance of CGRP (Table 2). Kaliuretic peptide did not affect the renal clearance of CGRP. Vessel dilator and LANP were still inhibiting ($P < .01$) the renal clearance of CGRP 3 hours after stopping their respective infusions. However, the amount of inhibition of renal clearance of CGRP 3 hours after ceasing LANP and vessel dilator infusion (1.7-fold and twofold, respectively) was less than the amount of inhibition of renal clearance of CGRP during the respective infusions. The inhibition of renal clearance of CGRP by ANF, on the other hand, was no longer present 90 minutes after stopping ANF infusion (Table 2). Thus, the ANF effects on the renal clearance of CGRP lasted only half as long ($P < .01$) as the inhibitory effects of LANP and vessel dilator on the renal clearance of CGRP.

Correlation of CGRP With Circulating Atrial Peptide Concentrations

Simultaneous measurement of each of the atrial peptides before, during, and after their respective infusions showed that each of the atrial peptides began to decrease immediately after stopping infusion and that ANF decreased to baseline within 90 minutes of stopping its infusion (Fig 5). Vessel dilator, LANP, and kaliuretic peptide were still significantly ($P < .05$) elevated 2 hours after their respective infusions. The circulating concentrations of vessel dilator and LANP had a temporal correlation (Spearman rank correlation coefficient, $-.70$ ($P = .04$) and $-.90$ ($P = .19$), respectively) with their ability to inhibit the excretion rate of CGRP into urine (Table 1). Spearman rank correlation coefficients of the circulating concentrations of LANP, vessel dilator, and ANF with the increase in CGRP within the circulation secondary to these atrial natriuretic peptides (Fig 3) were .88 ($P = .02$), .79 ($P = .06$), and .71 ($P = .11$), respectively. In contrast to LANP and vessel dilator, when the ANF concentration increased in the circulation (Fig 5) with a simultaneous increase in CGRP in the circulation (Fig 3), the excretion rate of CGRP into the urine was increasing (Table 1) rather than decreasing. ANF only decreased the excretion of CGRP into urine after the circulating concentration of CGRP had decreased, as observed in comparing Fig 3 with Table 1.

DISCUSSION

Vessel dilator, LANP, and kaliuretic peptide from the *N*-terminus of the ANF prohormone and ANF (28-aa carboxy terminus of prohormone) each significantly decreased systolic and diastolic blood pressure in the present investigation. When each of the atrial peptides were infused simultaneously with an increase in their respective concentrations, each except for kaliuretic peptide also increased the circulating concentration of CGRP, suggesting that a portion of their vasodilating effects may be mediated by CGRP. The kaliuretic peptide effect on blood pressure, on the other hand, does not appear to involve CGRP. With respect to the blood pressure–lowering effects of CGRP, its effects on diastolic blood pressure are no longer present within 20 minutes of stopping its infusion, whereas its effects on systolic blood pressure cease within 40 minutes of stopping its infusion.[4] Fisher et al[2] have shown that 220 pmol CGRP intravenously causes a maximal decrease in mean atrial pressure by 1 minute, with the decrease in blood pressure lasting for less than 5 minutes.[2] Thus, the CGRP effects on blood pressure are of relatively short duration.[2,4] Some of the short-term effects of the atrial peptides on blood pressure might be mediated by CGRP, since the blood pressure decrease

Table 1. Atrial Natriuretic Peptides Decrease the Excretion Rate (fmol/mL urine excreted/min) of CGRP

	Time (min)										
	30	60	80	100	120	150	180	210	240	270	300
Infusion		[↑	Infusion		]↓						
Control	205	202	206	210	208	214	220	220	215	210	205
LANP	200	196	138	122	124	108	135	105	108	149	115
Vessel dilator	212	208	155	154	182	99	110	103	96	110	130
Kaliuretic peptide	203	204	205	208	203	200	186	195	199	204	202
ANF	216	210	240	221	245	130	110	221	238	272	300

NOTE. Excretion rate is the concentration measured in urine times urine output (ie, flow in milliliters) divided by the number of minutes to produce the quantity of urine measured. Each of the respective atrial natriuretic peptides was infused at 100 ng/kg BW · min for 60 minutes from 60 to 120 minutes, with the decrease in the urinary excretion rate of CGRP postinfusion being significant at $P < .01$ for LANP, vessel dilator, and ANF when evaluated by repeated-measures ANOVA followed by MRT. Kaliuretic peptide did not significantly affect the excretion rate of CGRP. For each group, n = 6. The variation of mean values ranged from 3% to 10%.

Table 2. LANP, Vessel Dilator, and ANF Decrease the Renal Clearance (mL/min) of CGRP

	Time (min)										
	30	60	80	100	120	150	180	210	240	270	300
Infusion		[↑	Infusion		↑]						
Control	2.92	2.86	2.99	3.00	2.97	2.97	3.14	3.12	2.89	3.04	2.85
LANP	2.86	2.76	0.66	0.59	0.59	0.51	0.96	0.81	0.86	1.49	1.35
Vessel dilator	2.99	2.97	0.69	0.68	0.81	0.44	0.96	0.94	0.96	1.22	1.73
Kaliuretic peptide	2.90	2.91	2.93	2.89	2.90	2.86	2.74	2.79	2.84	2.91	2.89
ANF	3.00	2.96	3.16	0.76	0.88	0.65	1.57	3.16	3.05	3.44	3.80

NOTE. Renal clearance rate is the excretion rate that is the CGRP concentration measured in urine times urine output (ie, flow in milliliters) divided by the number of minutes to produce the quantity of urine measured, which in turn is divided by the measured plasma concentration of CGRP at each of the respective time points. Each of the respective atrial natriuretic peptides was infused at 100 ng/kg BW · min for 60 minutes from 60 to 120 minutes, with the decrease in the renal clearance rate of CGRP (mL/min) postinfusion being significant at $P < .01$ for LANP, vessel dilator, and ANF when evaluated by repeated-measures ANOVA followed by MRT. Kaliuretic peptide did not significantly affect the renal clearance rate of CGRP. For each group, n = 6. The variation of mean values ranged from 3% to 10%.

secondary to the respective atrial peptides correlated in a temporal fashion in the present investigation with their ability to increase CGRP.

However, the CGRP effects on blood pressure may not be direct. Arguing against the CGRP effects on blood pressure being direct but rather mediated by some other peptide (such as the atrial peptides) or some other substance is the fact that the vasodilation in vitro associated with CGRP requires an intact endothelium.[3] The required presence of intact endothelium for any blood pressure–lowering activity suggests that CGRP may be releasing something from the endothelium that in turn is causing the vasodilation. ANF, LANP, and vessel dilator are present in the endothelium as determined by both immunoperoxidase and immunofluorescent staining.[24,25] Thus, one peptide (ie, ANF) that CGRP increases in the circulation[4] is present in endothelium, and if CGRP also increases ANF (and/or the other atrial peptides) present within the endothelium, this would account for the blood pressure–lowering effects attributed to CGRP. It should be noted in this regard that LANP, ANF, and vessel dilator's vasodilating effects are direct effects that do not require endothelium to be present.[12,26] Since all of the blood pressure effects of CGRP have followed a temporal pattern of increasing ANF, it may be that its effects on blood pressure are mediated via atrial peptides, each of which have direct vasodilating effects in vitro,[12,26] rather than by CGRP itself.

CGRP has also been reported to increase sodium excretion and to cause a diuresis while at the same time increasing ANF in the circulation.[4] LANP, vessel dilator, and ANF also have potent natriuretic and diuretic effects, with urine flow increasing fourfold to 12-fold and sodium excretion threefold to 11-fold in the subjects of the present investigation during infusion of the respective atrial peptides.[11] The question thus arises as to whether the atrial peptides or CGRP is the final mediator of the diuretic and/or natriuretic effects that have been observed with these peptides. Insight into answering this question comes from the temporal association of the respective biologic effects, atrial peptides, and/or CGRP. When CGRP is infused for 10 minutes, ANF increases and reaches a maximal concentration at the end of the 10-minute infusion.[4] ANF decreases immediately after CGRP infusion and is completely back to baseline within 20 minutes of stopping the infusion.[4] With a CGRP infusion, sodium excretion follows identically the ANF pattern of increasing at 10 minutes and decreasing to one fifth of the increased excretion within 10 minutes of stopping the infusion.[4] Within 20 minutes of stopping CGRP infusion, sodium excretion returns to preinfusion levels.[4] Urinary volume also increases and is

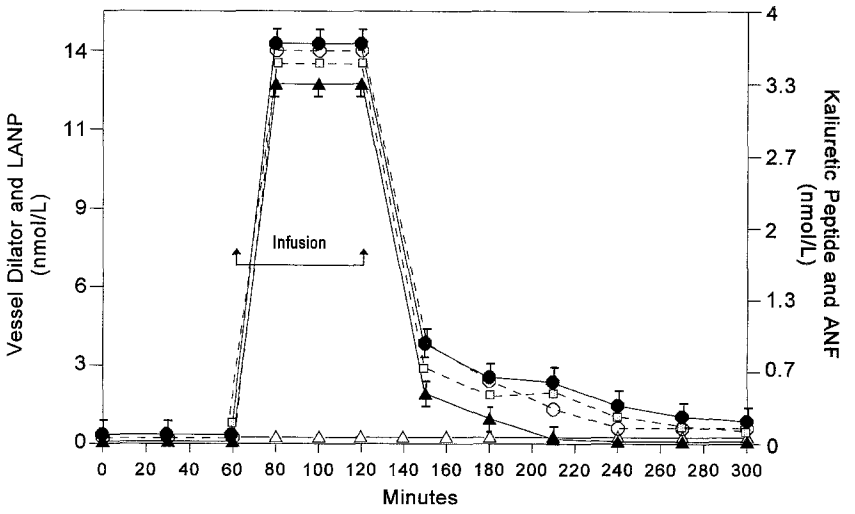

Fig 5. Increase of atrial natriuretic peptides within the circulation with their respective 100-ng/kg BW ± min for 60-minute infusions. LANP (○), vessel dilator (●), and kaliuretic peptide (□) were still significantly ($P < .05$) increased 2 hours after stopping their infusions, whereas ANF (▲) had returned to its preinfusion concentration within 90 minutes, when evaluated by ANOVA followed by MRT. Infusion of vehicle (△, 20 mL normal saline) did not cause the circulating concentration of any of these peptides to increase. Thus, the concentration of these peptides noted at 0, 30, and 60 minutes in the preinfusion periods remained constant (△) throughout the 300-minute experiment. n = 6 for each group.

maximal at the end of a 10-minute CGRP infusion.[4] Urinary volume decreases immediately upon stopping CGRP infusion and is less than half its peak volume within 10 minutes of cessation of CGRP infusion.[4] All of the diuresis associated with CGRP is completed within 40 minutes of stopping its infusion.[4] Since the diuresis and natriuresis secondary to vessel dilator, LANP, and kaliuretic peptide last for hours ($P < .01$) after they have returned to preinfusion concentrations,[11] it has been thought that these peptides have a long biologic activity. The present investigation would suggest an alternate hypothesis that the prolonged natriuresis and diuresis secondary to LANP and vessel dilator may be mediated in part by CGRP, for although the CGRP effects last less than 1 hour after it is increased in the circulation, these peptides increased CGRP for 2 hours after stopping their infusions (Fig 2), which would make the diuresis and natriuresis secondary to these atrial natriuretic peptides correlate in a temporal fashion with the increase in CGRP.

Arguing against all of the natriuretic and diuretic effects of vessel dilator and LANP being mediated by CGRP is the fact that part of their natriuretic mechanism of action involves inhibiting Na^+-K^+-ATPase within the kidney via their ability to increase the synthesis of prostaglandin E_2.[27,28] When an inhibitor of cyclooxygenase such as indomethacin is added in vivo, the natriuretic effect of vessel dilator is markedly attenuated,[29] similar to that found by using an inhibitor of prostaglandin synthesis in vitro simultaneously with vessel dilator.[27,28] The addition of indomethacin to CGRP infusions, on the other hand, has been reported to have no effect on the increased blood flow associated with CGRP.[3] Thus, if a cylooxygenase inhibitor does not modulate CGRP effects while the same cylooxygenase inhibitor markedly attenuates the natriuresis and diuresis secondary to vessel dilator, it would appear that the biologic effects of vessel dilator are not mediated by CGRP.

Since LANP and kaliuretic peptide also have a mechanism of action similar to that of vessel dilator of increasing the synthesis of prostaglandin E_2, resulting in inhibition of renal Na^+-K^+-ATPase, as their mechanism of action at the level of the kidney,[28] one would suspect that CGRP does not mediate their diuretic and/or natriuretic effects either. The ANF natriuretic effects, on the other hand, are not mediated by increasing prostaglandin E_2 synthesis with resultant Na^+-K^+-ATPase inhibition.[27,28] Thus, the ANF effects might be mediated by CGRP, since the increase of CGRP in the present investigation did correlate temporally with the natriuresis and diuresis secondary to ANF infusion. Arguing against CGRP's mediating ANF-induced natriuresis is the knowledge that at the cellular level the natriuresis secondary to ANF is mediated by cGMP.[30] When infused, cGMP itself can cause a natriuresis.[30] CGRP, on the other hand, increases cAMP rather than cGMP.[31] cAMP has never been shown to mediate any natriuretic effect and is associated with an antidiuretic effect rather than diuresis.[7] This knowledge would strongly suggest that CGRP does not mediate ANF-induced natriuresis, since it does not increase either the cyclic nucleotide (cGMP) or prostaglandin (prostaglandin E_2) that mediate natriuresis. This knowledge would further suggest that the natriuresis and diuresis associated with CGRP is not a direct effect of CGRP, but is rather at least partially due to its increasing ANF, which does have a direct effect through cGMP of causing a natriuresis and diuresis.

The increase in CGRP in the circulation secondary to LANP and vessel dilator was associated with a simultaneous decreased excretion of CGRP into urine, suggesting that the effects of these peptides on increasing CGRP in the circulation may involve inhibiting the metabolic breakdown of CGRP. (If an inhibition in metabolic breakdown as the cause of the CGRP increased plasma concentration were not occurring, one would have expected the urinary excretion rate not to have decreased but rather to have remained the same or increased.) The finding that LANP and vessel dilator decrease the renal clearance of CGRP and decrease its excretion rate suggests that LANP and vessel dilator effects on the clearance of CGRP occur within tubules of the kidney. This investigation is the first evidence that any atrial peptide can inhibit the metabolic processing and/or renal clearance of any other hormone. Although atrial peptides have previously been demonstrated to inhibit the release of other hormones,[7,10] they have not previously been shown to inhibit the metabolic breakdown and/or renal clearance of another hormone.

In contradistinction to the other atrial peptides, the ANF-induced decreased excretion of CGRP into the urine did not occur during its infusion when it increased CGRP in the circulation. Rather, the decreased excretion of CGRP into the urine secondary to ANF occurred during the hour after the infusion had ceased. This would suggest that the increase in CGRP in the circulation secondary to ANF was due to stimulation of CGRP release into the circulation rather than inhibition of the metabolic processing of CGRP. However, ANF did decrease the renal clearance of CGRP during the latter part of its infusion and for 1 hour after stopping its infusion, suggesting that in addition to the ANF inhibition of the release of CGRP, ANF may also affect renal clearance of CGRP in a delayed fashion. The lack of effect on the renal clearance of CGRP during infusion of kaliuretic peptide strongly suggests that this peptide has no effect on kidney tubules with respect to the clearance of CGRP. Thus, although ANF, vessel dilator, and LANP each increased CGRP in the circulation, their mechanisms of action were different, with LANP and vessel dilator inhibiting the renal clearance of CGRP, and ANF's early effects involving stimulation of the release of CGRP.

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