

Cardiovascular and Hormonal Effects of Calcitonin Gene-Related Peptide in Congestive Heart Failure

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The effects of infusing human alpha-calcitonin gene-related peptide were studied in eight patients with congestive heart failure, five normal rabbits and five rabbits with adriamycin-induced cardiomyopathy. In patients with heart failure, calcitonin gene-related peptide caused a dose-dependent increase in cardiac output and decrease in pulmonary and systemic vascular resistance and pulmonary artery pressure. The systemic blood pressure and right atrial and pulmonary wedge pressures decreased only at the highest infusion rate (16 ng/kg per min). Heart rate remained unchanged. Plasma epinephrine increased ($p < 0.05$), whereas aldosterone, atrial natriuretic peptide and prolactin concentrations decreased ($p <$

0.05). Plasma norepinephrine, renin activity, cortisol and growth hormone concentrations remained unchanged.

In both groups of rabbits, the drug decreased blood pressure and increased cardiac output and heart rate. There was a significant increase in renal blood flow ($p < 0.05$). The peptide did not affect the contraction amplitude of human and rabbit ventricular myocytes.

These findings suggest that calcitonin gene-related peptide is a vasodilator in the rabbit and humans with little direct effect on ventricular myocardium. This peptide may be useful in some forms of heart failure.

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Calcitonin gene-related peptides are a group of recently discovered regulatory neuropeptides with widespread distribution in humans and animals. These peptides are located in the central and peripheral nervous system (1-3), the heart and blood vessels (4,5). Rat and human alpha-calcitonin gene-related peptides have been synthesized and the human peptide is found circulating in blood at relatively high concentrations (6). In the heart and blood vessels, specific binding sites have been identified (7-9). Calcitonin gene-related peptide acts on these receptors to exert powerful cardiovascular effects, including positive chronotropic and inotropic actions on the heart, hypotension and vasodilation in humans and several animals (9-15). These effects are independent of beta-adrenergic and histaminergic receptors or blockade of prostaglandin synthesis. Calcitonin gene-related peptide stimulates the inward calcium current in the bullfrog heart, seemingly mediated by the stimulation of adenylate cyclase (16).

In contrast to the vasodilation produced by acetylcholine and sodium nitroprusside, which increase cyclic guanosine

monophosphate in the smooth muscle, the effect of calcitonin gene-related peptide is not accompanied by guanosine monophosphate accumulation (17) and the vasodilation is nonendothelium-dependent (18). Intravenous capsaicin stimulates the release of endogenous calcitonin gene-related peptide, causing vasodilation, hypotension and a depletion of the peptide from nerve fibers in the myocardium and around the coronary vessels (9,19). These findings suggest that endogenous calcitonin gene-related peptide may play a role in modulating coronary and peripheral vascular tone. Moreover, a combination of the reported positive inotropic effect on the heart and arterial vasodilation may be beneficial in the treatment of congestive heart failure.

In this study, we investigated the effects of infusing human alpha-calcitonin gene-related peptide on 1) hemodynamics and plasma hormone concentrations in patients with severe congestive heart failure; 2) hemodynamics and tissue blood flow in normal and cardiomyopathic rabbits; and 3) isolated myocytes from normal rabbits and patients undergoing cardiac transplantation or valvular surgery.

Methods

Human Experiments

Study patients. The human studies were carried out in eight patients admitted for the control of congestive heart failure; six were male and two female, with an average age of

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37 years (range 17 to 50). One had ischemic heart disease; all other patients had dilated cardiomyopathy. The diagnosis was confirmed by cardiac catheterization and coronary angiography. All patients were short of breath (New York Heart Association functional class III or IV). Edema was present in three patients; jugular venous pressure was raised in all and the liver was enlarged in seven. All patients had clinical cardiomegaly and a left ventricular third heart sound. Four patients had evidence of mitral regurgitation. The average cardiothoracic ratio was $62.4 \pm 2.6\%$ on chest X-ray study. All routine laboratory investigations, including plasma electrolytes, creatinine and blood urea nitrogen, were within the normal limits. Five patients were taking digoxin (0.25 mg/day), all were on an oral diuretic drug (average furosemide dose 57 ± 8 mg/day) and three patients were taking captopril (25 mg three times a day).

Hemodynamics. Hemodynamics were measured with a Swan-Ganz catheter. Arterial pressure was measured using intraarterial cannulation of the left brachial artery using a 3F teflon catheter (Seldicath). Pressures were measured with Hewlett-Packard model 1290C transducers and a Hewlett-Packard model 78354A monitor. Cardiac output was determined by thermodilution (SP1445, Gould Instruments). Hemodynamic variables were derived using standard formulas.

Hormone assay. Hormone assays were carried out in a 30 ml blood sample drawn from a forearm vein. Plasma norepinephrine and epinephrine were measured by high performance liquid chromatography with electrochemical detection. Plasma renin activity, atrial natriuretic peptide, aldosterone, cortisol, prolactin and growth hormone were measured by radioimmunoassay (20,21). Plasma concentrations of calcitonin gene-related peptide were measured by nonequilibrium radioimmunoassay employing a commercial kit (ITS, Immunotechnology Service Production), with a standard curve range of 2.5 to 250 pg/ml. Plasma samples were assayed after a simple solid-phase extraction ($\text{Sc}_2\text{Pak C}_{18}$ cartridges). Analytical recovery of synthetic calcitonin gene-related peptide (128 pg/ml) added to plasma before extraction was 60% to 70% (mean 67%; $n = 10$). The mean concentration of circulating calcitonin gene-related peptide in human extracted plasma from 20 normal subjects was 11.3 ± 4.7 pg/ml (range 2.5 to 19.6).

Protocol. The studies were performed in the postabsorptive state during the afternoon, 3 h after a light lunch and 6 h after the last cardiac medication. After instrumentation, patients were allowed to rest in bed for 1 h. After baseline measurements, a blood sample was taken from a peripheral vein for estimation of basal hormone levels. Infusion of human alpha-calcitonin gene-related peptide (Celltech Ltd.) was then started into a forearm vein using an infusion pump (Harvard Apparatus, model 902). Incremental infusions of calcitonin gene-related peptide at the dose of 0.8, 3.2 and 16 ng/kg per min were made (2.16×10^{-13} , 8.3×10^{-13} and 4.2×10^{-12} mol/kg per min). The first two doses were infused for 10 min each. At the end of each 10 min infusion

period, all hemodynamic measurements were recorded. The highest dose of calcitonin gene-related peptide (16 ng/kg per min) was infused for 20 min. At the end of this period, hemodynamic measurements were repeated and a second blood sample for hormone assay was taken from a peripheral vein of the contralateral arm to that being infused. The infusion was then stopped. Hemodynamic measurements were continued for 1 h.

The human studies were performed after obtaining written informed consent from the patients. Ethical clearance was obtained from the Hospital Ethics Committee. The animal experiments were in conformity with the position of the American Heart Association on Research Animal Use and was cleared by the Hospital Ethics Committee.

Animal Experiments

Study animals. Experiments were carried out in two groups of five New Zealand White rabbits, weighing 1.9 to 3.1 kg. The first group of five control rabbits received no treatment. The second group of five animals received adriamycin into an ear vein at a dose of 1 mg/kg body weight, twice weekly for 7 weeks. We (22) previously showed this treatment to cause cardiomyopathy in rabbits. The adriamycin-treated animals were allowed a 2 week injection-free period to recover from the hematologic and renal toxicity. Polyethylene catheters were then implanted into the right atrium, left ventricle and ascending aorta. A specially designed thermistor catheter was inserted into the left femoral artery and advanced into the descending aorta. This was used for determining cardiac output by thermodilution (22). The surgical technique has been described in detail (22).

Protocol. Forty-eight hours after surgery, the rabbits underwent hemodynamic study in the conscious unrestrained state. Control measurements of heart rate, right atrial pressure, mean aortic pressure, cardiac output and regional blood flow were made. Human alpha-calcitonin gene-related peptide was infused into the right atrium using an infusion pump. The infusion was begun at 1×10^{-11} mol/kg per min. Heart rate, aortic pressure and cardiac output were measured every 2 min. The infusion rate was increased every 5 min until cardiac output increased by approximately 25%. The final dose was infused for 10 min before hemodynamic measurements were again taken and a second determination of regional blood flow made using different radiolabeled microspheres. The average final dose for the control animals was 4.06×10^{-10} mol/kg per min compared with 5.8×10^{-10} mol/kg per min for the adriamycin-treated animals ($p > 0.05$).

The animals were killed using an excess of intravenous pentobarbitone. The heart, brain, kidney, spleen and small samples of small gut, skin and soleus and quadriceps muscles were removed, weighed and processed for radioactive counting to calculate the tissue blood flow and vascular resistance values (22). Gadolinium-153- and Scandium-46-labeled microspheres (New England Nuclear Ltd.) were

Table 1. Hemodynamic Changes With Infusion of 0.8, 3.24 and 16 ng/kg per min of Calcitonin Gene-Related Peptide (CGRP) in Eight Patients With Congestive Heart Failure

	CGRP Infusion (ng/kg per min)				1 h Postinfusion
	Basal	0.8	3.24	16	
Heart rate (beats/min)	104.6 ± 17.5	101.8 ± 16.5	103.0 ± 17.3	105.1 ± 17.0	103.6 ± 10.4
Right atrial pressure (mean mm Hg)	9.1 ± 5.2	9.3 ± 5.2	8.6 ± 5.6	6.8 ± 4.4*	7.6 ± 4.6
Pulmonary artery pressure (mean mm Hg)	38.4 ± 5.9	37.2 ± 9.4	36.5 ± 9.8†	29.4 ± 10.4‡	34.9 ± 12.0
Pulmonary artery wedge pressure (mean mm Hg)	23.5 ± 6.4	22.6 ± 6.0	21.7 ± 4.9	13.5 ± 6.0‡	19.8 ± 6.4†
Systemic arterial pressure (mean mm Hg)	83.9 ± 13.1	83.5 ± 13.8	83.0 ± 15.3	66.6 ± 12.4‡	78.2 ± 8.9
Cardiac index (liters/min per m ²)	1.6 ± 0.27	1.7 ± 0.28†	2.0 ± 0.32‡	2.74 ± 0.53*	2.01 ± 0.55†
Stroke volume index (ml beats per m ²)	15.9 ± 3.6	17.3 ± 4.3†	20.2 ± 4.5‡	26.2 ± 3.7*	19.5 ± 4.7†
Pulmonary vascular resistance (dynes·cm ⁻⁵)	470 ± 194	433 ± 227	372 ± 224‡	284 ± 140*	364 ± 217
Systemic vascular resistance (dynes·cm ⁻⁵)	2,374 ± 309	2,187 ± 263‡	1,907 ± 330‡	1,136 ± 192*	1,831 ± 373†

*p < 0.001. †p < 0.05. ‡p < 0.005 compared with basal values. Data are mean ± SD.

used (unless stated otherwise) because of their relatively long half-life, different energy spectrum and ease of separation in a gamma counter.

Isolated Cardiac Myocytes

Protocol. The direct myocardial effects of calcitonin gene-related peptide were determined in isolated rabbit and human myocytes. Myocytes were isolated from three normal rabbit hearts by Langendorff perfusion with a low calcium solution (9 $\mu\text{mol/liter}$), followed by a 2 min perfusion with solution containing collagenase and hyaluronidase (23). Human ventricular myocardial cells were obtained from the explanted hearts from two patients with coronary artery disease (left ventricle from a 48 year old man and right ventricle from a 63 year old man) and from the left ventricle of a 62 year old woman at the time of mitral valve replacement. Pieces of ventricle (100 mg to 3 g) were quickly chopped into cubes of approximately 1 mm³ using an array of razor blades. The ventricles were incubated for a total of 12 min at 35°C in 25 to 50 ml of the low calcium medium containing 1 to 2 $\mu\text{mol/liter}$ calcium. The medium was changed three times during this period, and the tissue was stirred by bubbling with 100% oxygen. The low calcium medium was removed by straining with 330 $\mu\text{mol/liter}$ calcium added, followed by two 45 min periods with the protease (pronase) and 30 $\mu\text{mol/liter}$ calcium added, followed by two 45 min periods with the protease omitted and 400 IU/ml of collagenase added. The medium was shaken gently throughout the incubation and kept under an atmosphere of 100% oxygen.

At the end of each 45 min period, the solution containing the dispersed cells was filtered through a 300 μm gauze and centrifuged at 40 g for 1 to 2 min. Isolated cells were superfused with a modified Krebs-Henseleit solution containing calcium (1 mmol/liter) equilibrated with 95% oxygen and 5% carbon dioxide at 32 ± 0.5°C and electrically stimulated (at 0.5 Hz, 5 ms, suprathreshold voltage). The contraction amplitude of the myocytes was measured in a perspex chamber, using a video camera edge detection system linked

to an inverted microscope (23). The spatial resolution of the system was 1 in 256 and the time resolution 20 ms. Contraction was expressed as percent cell shortening.

In the rabbit myocytes, the effects of 1×10^{-9} to 1×10^{-7} mol/liter of calcitonin gene-related peptide on the percent cell shortening was investigated cumulatively. In four human cells, the effect of 1×10^{-7} mol/liter of calcitonin gene-related peptide was measured, whereas in two other cells, the effects of 10^{-5} and 10^{-6} mol/liter of calcitonin gene-related peptide were investigated. Each concentration of calcitonin gene-related peptide was in contact with the cells for 5 min. At the end of the experiment, the effect of calcium (8 mmol/liter) was also investigated.

Statistics. Data are expressed as mean ± standard deviation unless stated otherwise. The probability of the difference between paired and unpaired data was calculated by Student's *t* test.

Results

Human Experiments

Hemodynamics (Table 1). The rest basal hemodynamic data in the eight patients showed severe cardiac dysfunction as judged by reduced cardiac output (1.6 ± 0.27 liters/min per m²) and increased pulmonary artery wedge pressure (23.5 ± 6.4 mm Hg) and pulmonary and systemic vascular resistance (470 ± 194 and 2,374 ± 309 dynes/cm², respectively). The lowest dose of calcitonin gene-related peptide (0.8 ng/kg per min) caused a small (7.9%) decrease in systemic vascular resistance (p < 0.005) and a similar increase (7%) in cardiac output (p < 0.05) (Fig. 1 and 2). The heart rate did not change. Calcitonin gene-related peptide at the dose of 3.24 ng/kg per min caused a further reduction in systemic vascular resistance (20%, p < 0.002) and increase in cardiac output (24%, p < 0.002). At this dose, pulmonary vascular resistance also decreased (71%, p < 0.004) and there was a slight decrease in the mean pulmonary arterial pressure (5%, p < 0.05). Systemic blood pressure did not change.

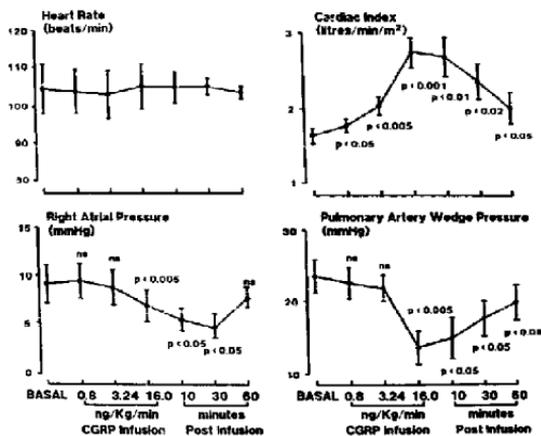


Figure 1. Hemodynamic data before, during and after (Post) calcitonin gene-related peptide (CGRP) infusion in eight patients with congestive heart failure.

At the highest dose of calcitonin gene-related peptide (16 ng/kg per min), systemic and pulmonary vascular resistance decreased further (52% $p < 0.001$ and 40% $p < 0.001$, respectively). Cardiac output increased by 67% ($p < 0.001$). Arterial pressure decreased by 21% ($p < 0.004$) and pulmonary arterial and pulmonary artery wedge pressures de-

creased by 21% ($p < 0.01$) and 42% ($p < 0.005$), respectively; right atrial pressure also decreased (24%, $p < 0.001$). Even at the highest dose of the peptide, heart rate did not change despite a significant decrease in systemic arterial pressure.

After the infusion was discontinued, hemodynamics

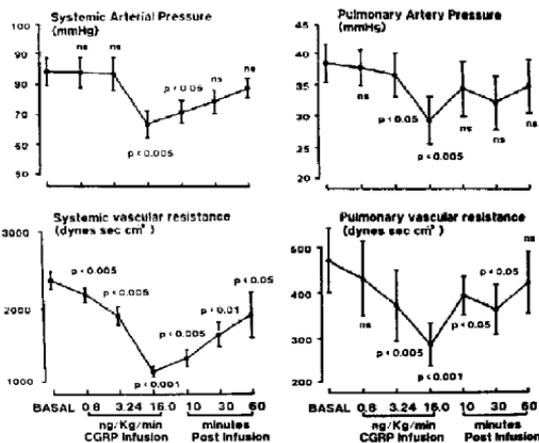


Figure 2. Hemodynamic data before, during and after (Post) calcitonin gene-related peptide (CGRP) infusion in eight patients with congestive heart failure.

Table 2. Plasma Hormone Levels Before and During Infusion of 16 ng/kg per min of Calcitonin Gene-Related Peptide (CGRP) in 8 Patients With Congestive Heart Failure Compared With Values in 16 Normal Control Subjects

	Normal Control Subjects	Patients (basal)	p Value (control vs. basal)	Patients During CGRP	p Value (basal vs. CGRP)
Norepinephrine (pg/ml)	275 ± 34	790 ± 258	<0.05	755 ± 163	NS
Epinephrine (pg/ml)	8 ± 16	119 ± 17	NS	323 ± 137	<0.05
Renin (ng/ml per h)	0.55 ± 0.16	4.0 ± 1.8	<0.01	4.76 ± 1.98	NS
Aldosterone (ng/ml)	92 ± 7	366 ± 135	<0.001	263 ± 138	<0.02
ANP (pg/ml)	22 ± 5	383 ± 45	<0.0001	250 ± 35	<0.005
Cortisol (ng/ml)	74 ± 12	166 ± 24	<0.0001	179 ± 22	NS
Growth hormone (ng/ml)	0.75 ± 0.16	6.0 ± 2.1	<0.005	3.59 ± 0.62	NS
Prolactin (ng/ml)	7.28 ± 0.88	11.6 ± 7.1	NS	4.59 ± 2.1	<0.02
CGRP (pg/ml)	11.35 ± 4.7	36.82 ± 27.6	<0.05	185.9 ± 127.2	<0.001

Data are mean ± SEM. ANP = atrial natriuretic peptide.

slowly returned toward the basal values. However, even 1 h after the infusion had been stopped, cardiac output was significantly higher ($p < 0.05$) and pulmonary wedge pressure and systemic vascular resistance significantly ($p < 0.05$) lower than the basal values. There were no complications or side effects during the infusion. In particular, none of the patients complained of flushing of the face or headache and no patient developed any arrhythmias.

Plasma hormones (Table 2). Plasma hormone levels before the start of the peptide infusion and at the end of 20 min at 16 ng/kg per min are shown in Table 2. Basal plasma norepinephrine levels were consistently raised. Although there was considerable individual variation, the average was nearly three times that of control subjects ($p < 0.05$). In contrast, epinephrine levels were within normal limits. Plasma renin activity varied greatly, although the mean value was seven times that of control subjects ($p < 0.01$). Aldosterone and cortisol levels also varied widely. Plasma atrial natriuretic peptide concentration was raised in every patient ($p < 0.001$). Growth hormone level was raised in all but one patient ($p < 0.005$). Serum prolactin levels were within the normal range. The average basal level of calcitonin gene-related peptide was increased in the patients (range 12.5 to 82.4 pg/ml, mean 36.82 ± 27.6). There were, however, two patients who had levels within the normal range, the values in our normal control subjects being 11.35 ± 4.7 pg/ml (range 2.5 to 19.6). Calcitonin gene-related peptide levels increased to five times the basal value (185.9 = 127.2 pg/ml) after infusion at the highest dose.

Calcitonin gene-related peptide infusion had no effect on plasma norepinephrine. Plasma epinephrine increased significantly from 119 ± 17 to 323 ± 137 pg/ml ($p < 0.05$). There was no consistent change in plasma renin activity. Plasma aldosterone decreased in all but one patient; the mean level decreased from 366 ± 135 to 263 ± 138 pg/ml ($p < 0.02$). Atrial natriuretic peptide decreased in every case; the average value decreased from 385 ± 45 to 250 ± 35 pg/ml ($p < 0.005$). Plasma growth hormone and cortisol did not change. The prolactin levels

decreased in all patients; the average level decreased from 11.6 ± 2.1 to 4.59 ± 2.1 ng/ml ($p < 0.02$).

Animal Experiments

Hemodynamics and tissue blood flow (Tables 3 and 4). Seven weeks of adriamycin treatment caused the development of cardiomyopathy in all five rabbits as judged by a significant reduction in cardiac output ($p < 0.05$), increase in the total peripheral resistance ($p < 0.05$) and a redistribution of tissue blood flow (Table 3).

Calcitonin gene-related peptide caused a 17% increase in heart rate in the five control animals ($p < 0.05$) (Table 3, Fig. 3). Although heart rate increased by 10% in the adriamycin-treated group it did not reach a level of significance. Mean blood pressure was reduced by about 30% in both groups ($p < 0.05$). There was a significant increase in cardiac output ($p < 0.05$), the effect being +31.2% in the control as compared with +24.5% in the adriamycin-treated animals. Total peripheral resistance decreased significantly by about the same amount in both groups. Stroke volume index increased in both groups, but the difference reached a significant level only in the control group.

Calcitonin gene-related peptide caused a marked increase in renal blood flow in both groups, but the effect was more striking in the adriamycin-treated group. Myocardial and skin blood flow was increased by calcitonin gene-related peptide, but the changes did not reach significance in either of the groups. Cerebral blood flow remained unaltered and there was no consistent change in splenic, small gut or skeletal muscle blood flow. The effect on the calculated tissue vascular resistance is shown in Table 4. Regional vascular resistance generally reflected changes in the tissue blood flow. However, cerebral and myocardial vascular resistance in both groups and the skin resistance in the control group decreased significantly ($p < 0.05$), but these were not accompanied by significant changes in tissue blood

Table 3. Effect of Calcitonin Gene-Related Peptide on the Hemodynamics and Tissue Blood Flow in Five Control and Five Adriamycin-Treated Rabbits

	Control		Adriamycin-Treated	
	Pre-drug	Post-drug	Pre-drug	Post-drug
Hemodynamics				
Heart rate (beats/min)	273.6 ± 20.7	321.0 ± 20.1*	286 ± 40	315.0 ± 21.2
Mean systemic pressure (mm Hg)	90.4 ± 17.2	61.2 ± 6.9*	96.2 ± 15.1	63.4 ± 15.9*
Right atrial pressure (mm Hg)	0.4 ± 0.5	0.3 ± 0	0.0 ± 0.8	1.0 ± 1.4
Cardiac output (ml/min per kg)	342.2 ± 96.0	443 ± 137*	230.2 ± 43.2†	286.6 ± 58.7*
Stroke volume index (ml/beat per kg)	1.26 ± 0.38	1.38 ± 0.35*	0.80 ± 0.177†	0.91 ± 0.97
Total peripheral resistance (mm Hg ml/min per kg)	0.277 ± 0.12	0.146 ± 0.04*	0.43 ± 0.092†	0.22 ± 0.24†
Tissue blood flow (ml/min per kg)				
Myocardial	5.91 ± 1.94	7.25 ± 2.82	8.7 ± 5.7	10.1 ± 3.55
Cerebral	1.134 ± 0.284	1.060 ± 0.302	0.71 ± 0.16	0.76 ± 0.35
Renal	4.26 ± 1.72	4.83 ± 1.58*	2.78 ± 1.38†	4.19 ± 1.98*
Splenic	6.57 ± 6.97	8.82 ± 7.25	2.86 ± 1.44	2.77 ± 0.78
Small gut	1.7 ± 1.04	2.1 ± 0.8	1.15 ± 0.56	1.41 ± 0.73
Soleus	0.31 ± 0.23	0.26 ± 0.25	0.18 ± 1.27	0.3 ± 0.4
Quadriceps	0.074 ± 0.016	0.052 ± 0.03	0.076 ± 0.63	0.58 ± 6.054
Skin	0.136 ± 0.04	0.27 ± 0.18	0.052 ± 0.027†	0.072 ± 0.059

*p < 0.05, †p < 0.01 compared with pre-drug levels; ‡p < 0.05 adriamycin-treated and control rabbits before drug treatment. Data are mean ± SD.

flow to those organs. In addition, renal vascular resistance decreased significantly in the adriamycin-treated rabbits.

Isolated Cardiac Myocytes

Contraction amplitude. No increase in contraction amplitude was seen in either the seven rabbit myocytes (three rabbits) or in the four human cells tested (Fig. 4). The concentration of calcitonin gene-related peptide was increased to 10^{-7} mol/liter in all cells and to 10^{-5} mol/liter in two of the human myocytes. Every cell subsequently tested showed increased contraction amplitude in response to raised extracellular calcium, indicating that it had the potential to contract more strongly. The contraction amplitude in a maximally stimulating concentration of calcium (8 mmol/liter in rabbit, 6 to 15 mmol/liter in human) is shown in Figure 4.

Discussion

In this study, we report for the first time the effects of infusions of human calcitonin gene-related peptide on the hemodynamics and plasma hormone concentrations in patients with severe congestive heart failure. In addition, we studied the hemodynamic and regional blood flow in groups of normal and adriamycin-treated rabbits. To test whether calcitonin gene-related peptide has any direct inotropic effects on the heart, we examined its effects on isolated cardiac myocytes.

Human Study

Vasodilating effects. In patients with congestive heart failure, infusion of calcitonin gene-related peptide caused a dose-dependent decrease in systemic and pulmonary vascular resistance and an increase in cardiac output. The consid-

Table 4. Effect of Calcitonin Gene-Related Peptide on Tissue Vascular Resistance (dynes·s·cm⁵/kg) in Five Control and Five Adriamycin-Treated Rabbits

	Control		Adriamycin-Treated	
	Pre-drug	Post-drug	Pre-drug	Post-drug
Myocardial	12.46 ± 3.66	5.26 ± 2.51*	9.07 ± 4.4	4.67 ± 2.84*
Cerebral	85.0 ± 31.6	65.1 ± 25.9*	141.6 ± 41.1	92.3 ± 14.5†
Renal	25.61 ± 19.4	14.56 ± 6.77	43.3 ± 22.8†	18.8 ± 10.13*
Splenic	64.8 ± 106.5	41.7 ± 75.5	39.3 ± 69.8	23.5 ± 4.4
Small gut	291.0 ± 516	33.35 ± 15.1	101.3 ± 50.5	49.4 ± 15.2
Soleus	436.0 ± 243	526.0 ± 527	5,800 ± 11,991	3,536 ± 4,470
Quadriceps	1,277 ± 433	1,586 ± 1,074	12,238 ± 23,672	6,688 ± 12,211
Skin	360 ± 399	314 ± 203*	2,353 ± 1,304†	1,895 ± 2,187

*p < 0.05, †p < 0.01 compared with pre-drug levels; ‡p < 0.05 adriamycin-treated and control rabbits before drug treatment. Data are mean ± SD.

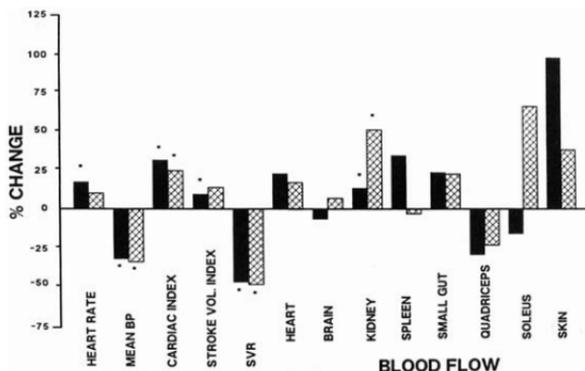


Figure 3. Percent change in hemodynamic and tissue blood flow after infusion of human alpha-calcitonin gene-related peptide (CGRP) in five normal rabbits (solid bars) and five rabbits with adriamycin-induced cardiomyopathy (cross-hatched bars). BP = blood pressure; SVR = systemic vascular resistance; VOL. = volume. * $p < 0.05$.

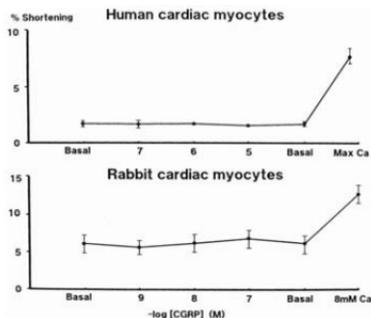
erale increase in cardiac output probably prevented a decrease in blood pressure until the highest dose (16 ng/kg per min) was reached. The two lower doses of the peptide did not cause any change in the left- or right-sided filling pressures of the heart. At the highest dose, both the right atrial and pulmonary arterial wedge pressure decreased. This would suggest that the peptide behaves as an arteriolar dilator at low doses and a mixed vasodilator at high doses. Recently, McEwan et al. (24) showed dilation of the forearm resistance vessels in humans in response to direct brachial artery infusion of calcitonin gene-related peptide. Maximal increase in forearm blood flow was obtained with infusion of 1.2 to 10×10^{-12} mol/min of calcitonin gene-related peptide (4 to 40 ng/min). They were unable to show any venodilation

with infusion of up to 5×10^{-12} mol/min of the peptide (20 ng/min) directly into the vein. This lack of effect on the capacitance vessels, in contrast to a venodilator effect seen in our patients with the highest dose, may be due to the higher rest venous tone in patients with congestive heart failure or the higher final dose used in our patients.

Role of tachycardia. In normal human volunteers, intravenous calcitonin gene-related peptide has been shown to cause tachycardia (6,12,13,25), with either no change (25) or a decrease in systemic blood pressure (6,12,13). The lack of reflex tachycardia in our patients even at the highest dose that caused a decrease in blood pressure is an interesting observation. Such a response might occur when improvement in hemodynamics is accompanied by a withdrawal of sympathetic overactivity or it might reflect the baroreflex abnormalities seen in patients with congestive heart failure (26). Conversely, the absence of a tachycardia response might be the result of a specific effect of this peptide on the sinus node. Ono et al. (16) have shown that the chronotropic effects of calcitonin gene-related peptide on the heart are mediated through specific receptors that increase transmembrane calcium current (10^{-8} to 10^{-7} mol/liter). In the presence of the catecholamine isoproterenol, calcitonin gene-related peptide has the opposite effect and blocks the isoproterenol-stimulated calcium current. It is likely that in patients with congestive heart failure, the combination of high circulating levels of catecholamines and calcitonin gene-related peptide block the development of tachycardia.

Peptide binding sites. Specific binding sites for calcitonin gene-related peptide have been identified in the heart and blood vessels (9). In the rat, the highest concentrations of binding sites for calcitonin gene-related peptide are in the right atrium, followed by the left atrium and right and left ventricles. Positive inotropic effects of calcitonin gene-related peptide seen in *in vitro* studies (9,27) are probably

Figure 4. Percent shortening of human and rabbit cardiac myocytes after treatment with various doses of calcitonin gene-related peptide (CGRP) and calcium (Ca). Max = maximal.



brought about by direct interaction of calcitonin gene-related peptide with these receptors and are not influenced by adrenergic, histaminergic and prostaglandin synthesis blockade. In most species, the positive inotropic effects of calcitonin gene-related peptide are seen in the atria and not in the ventricle (10,14,15). In humans, the positive inotropic effect of the peptide could be suppressed by labetalol, an alpha- and beta-adrenoceptor blocker (12), which suggests that the inotropic effect could have been caused by reflex sympathetic stimulation. The effect of calcitonin gene-related peptide on isolated myocytes has never been reported. The lack of any *in vitro* inotropic effect seen in our isolated ventricular myocytes suggests that specific binding sites for this peptide may not be present in the human and rabbit ventricles. The increase in cardiac output seen in our patients and rabbits after calcitonin gene-related peptide infusion is, therefore, most likely due to altered hemodynamics and, in particular, the decrease in afterload.

Hormone Studies

Plasma peptide concentration. The concentrations of plasma hormones in our patients with congestive heart failure are similar to those described previously (28). In normal humans, relatively high concentrations of circulating immunoreactive calcitonin gene-related peptide have been reported (25 to $941 \pm 34.2 \times 10^{-12}$ mol/liter) (6,29). We measured calcitonin gene-related peptide using an extraction procedure followed by radioimmunoassay and obtained a mean value of 11.3 ± 4.7 pg/ml, corresponding to 3×10^{-12} mol/liter, much lower than the concentrations described by others (6,29). Although the concentration of the peptide was increased in most patients with congestive heart failure, there was considerable individual variation. There was no correlation between the calcitonin gene-related peptide concentration and baseline hemodynamics or hormone levels. The increased level of the peptide in patients with congestive heart failure has not been reported previously. The pathophysiologic significance is not clear, but may reflect a role of this peptide in congestive heart failure.

Plasma epinephrine and norepinephrine. Calcitonin gene-related peptide infusion caused a significant increase in plasma epinephrine and decrease in aldosterone, atrial natriuretic peptide and prolactin concentrations. An increase in plasma epinephrine and norepinephrine levels after calcitonin gene-related peptide infusion has been described by others (6,13) and may be related to the decrease in systemic blood pressure and reflex sympathetic activation. Norepinephrine did not increase in our patients. This dissociation between epinephrine and norepinephrine levels is unusual and may suggest a direct effect of the peptide on the adrenal medulla. A reduction in atrial natriuretic peptide seen in every patient was probably due to a decrease

in the filling pressures seen with calcitonin gene-related peptide.

Plasma renin and prolactin. Kurtz et al. (30) administered calcitonin gene-related peptide to normal human volunteers in doses similar to those used by us and found an increase in plasma renin activity. Plasma aldosterone did not change. They examined the effect of calcitonin gene-related peptide on isolated juxtaglomerular cells and concluded that the increase in renin secretion was the result of a direct regulatory influence on the juxtaglomerular cells and not necessarily due to a decrease in blood pressure. In our patients, short-term infusion of calcitonin gene-related peptide did not change renin activity, although the plasma aldosterone concentration decreased. The differences in these findings cannot easily be explained. Calcitonin gene-related peptide infusion caused a significant decrease in prolactin levels in our patients. Although the effect of this peptide on prolactin has not been reported before, calcitonin, which has structural homology with calcitonin gene-related peptide, has been shown to exert an inhibitory effect on prolactin secretion in patients with impaired renal function (31).

Animal Study

Adriamycin cardiomyopathy. We have previously shown (22) that the adriamycin treatment used in this study leads to the development of cardiomyopathy, with reduced cardiac output at rest, a flat Frank-Starling curve, increased total body exchangeable sodium and a redistribution of regional blood flow. This model of low output failure has been successfully applied to study the effect of drugs in the conscious animal at rest (32). The hemodynamic and regional blood flow alterations observed in the adriamycin-treated animals in this study resembled those reported by us previously (22,32) and are similar to the changes seen in patients with heart failure (33,34).

Hemodynamic effects. In the normal rabbits, calcitonin gene-related peptide in doses that caused hypotension resulted in an increase in cardiac output, heart rate and renal blood flow. Qualitatively similar hemodynamic and tissue blood flow changes were seen in the rabbits with heart failure. *In vitro* studies have shown considerable variation in the effects of calcitonin gene-related peptide in different species. Thus, whereas it has positive chronotropic and inotropic effects on isolated rat and guinea pig atria, it does not alter the rate or force of contraction in the isolated rabbit heart (10,11). In our study, there was only a modest increase in heart rate in the normal rabbits and no change in the rabbits with heart failure despite a considerable decrease in blood pressure.

Effects on renal and peripheral blood flow. Calcitonin gene-related peptide increased renal blood flow and reduced renal vascular resistance in both groups of animals. The renal effects of this peptide, unlike those of atrial natriuretic peptide, have not been well studied, but an increase in renal

blood flow has been shown to occur in the rat (35). The effect of calcitonin gene-related peptide on other vascular beds was not so impressive. This may be in part due to technical factors. Skin and muscle blood flow is very small, so that the reliability of radioactivity counting is reduced. Thus, the substantial percent increase in skin blood flow after calcitonin gene-related peptide did not reach a level of significance, whereas the decrease in resistance was significant only in the control animals. These limiting technical factors do not apply to the brain, where blood flow is considerable and clearly did not significantly change during infusion of calcitonin gene-related peptide. However, the maintenance of blood flow in the presence of a reduced perfusion pressure implies a degree of vasodilation. Furthermore, there was a significant decrease in resistance in the rabbits with cardiomyopathy, which is consistent with the presence of calcitonin gene-related peptide receptors in the cerebral arteries (7,8).

Conclusion. The present study confirms that calcitonin gene-related peptide exerts powerful pharmacologic effects on the cardiovascular and endocrine systems, but the physiologic significance of these effects remain to be determined. The peptide may act directly through its specific receptors and indirectly by influencing the release and action of other cardiovascular transmitters. Exogenous calcitonin gene-related peptide produced beneficial hemodynamic effects during short-term infusions in patients with severe congestive heart failure and were attributable to vasodilation and not a positive inotropic effect. As yet, there is no report of the development of tachyphylaxis after prolonged infusion. These cardiovascular actions of calcitonin gene-related peptide along with its capacity to increase renal blood flow suggest a useful role in clinical cardiology.

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