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Direct Vasoactive Effects of the Chromogranin A (CHGA) Peptide Catestatin in Humans *In Vivo*

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Abstract

Catestatin is a bioactive peptide of chromogranin A (CHGA) that is co-released with catecholamines from secretory vesicles. Catestatin may function as a vasodilator and is diminished in hypertension. To evaluate this potential vasodilator *in vivo* without systemic counterregulation, we infused catestatin to target concentrations of ~ 50, ~ 500, ~5000 nM into dorsal hand veins of 18 normotensive men and women, after pharmacologic venoconstriction with phenylephrine. Pancreastatin, another CHGA peptide, was infused as a negative control. After precontraction to ~ 69%, increasing concentrations of catestatin resulted in dose-dependent vasodilation ($P = 0.019$), in female subjects (to ~ 44%) predominantly. The EC_{50} (~ 30 nM) for vasodilation induced by catestatin was the same order of magnitude to circulating endogenous catestatin (4.4 nM). No vasodilation occurred during the control infusion with pancreastatin. Plasma CHGA, catestatin, and CHGA-to-catestatin processing were then determined in 622 healthy subjects without hypertension. Female subjects had higher plasma catestatin levels than males ($P = 0.001$), yet lower CHGA precursor concentrations ($P = 0.006$), reflecting increased processing of CHGA-to-catestatin ($P < 0.001$). Our results demonstrate that catestatin dilates human blood vessels *in vivo*, especially in females. Catestatin may contribute to sex differences in endogenous vascular tone, thereby influencing the complex predisposition to hypertension.

Keywords

catestatin; chromogranin A; vasodilation; veins

INTRODUCTION

An altered adrenergic system has been implicated in hypertension. The neurotransmitters of this system, the catecholamines, are stored and released from vesicles in neuroendocrine cells and neurons. Soluble secretory proteins, such as chromogranin A (CHGA), catalyze the formation of the secretory vesicles (1,2) and are co-released with catecholamines when

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stimulated. Chromogranin A is heritable and elevated in essential hypertension (3). Through post-translational proteolytic processing, CHGA gives rise to a number of bioactive peptides (4), including catestatin, human CHGA_{352–372}, which has cardiovascular effects in animal studies (5–8). Details of the post-translational processing have not been clearly elucidated but appear to occur both intracellularly and extracellularly (9–10) and involve enzymes such as the serine protease plasmin and the cysteine protease cathepsin L. (11,12). A *Chga* knockout mouse (*Chga* $-/-$) had elevated blood pressure (BP), which substantially improved towards the wild-type value with the infusion of catestatin (2). In humans, plasma levels of catestatin are diminished not only in hypertensive patients but also in their still-normotensive offspring (13). Human genetic variants may also affect autonomic activity and alter hypertension risk (14).

In this study, we infused catestatin into a human local vascular bed *in vivo* to evaluate whether it acts as a vasodilator, then probed basal catestatin levels in a normotensive population to better understand the potential impact of catestatin on BP.

MATERIALS AND METHODS

Dorsal Hand Vein Subjects

We studied 18 ambulatory, normotensive men and women (aged 19–51 y). Family history of hypertension (history of high BP before the age of 60 y in a first degree relative) was determined by self-report in 67% of subjects. Biogeographic ancestry was self-classified. Regular prescription medication use was reported in two subjects, one female taking hormone replacement therapy, and another taking acyclovir and baclofen as needed for oral herpes and back spasms. Three of the female subjects were post-menopausal at the time of the study. Subjects were requested to avoid alcohol and caffeine for at least 12 h prior to participation and were nonfasting but encouraged to continue their standard fluid intake. All female subjects had a negative urine pregnancy test on the day of the study.

The study protocol was approved by the Human Research Protection Program of the University of California at San Diego. Written informed consent was obtained from each subject.

Measurement of Vascular Responses

Local venous responses were measured in a cannulated dorsal hand vein using a linear variable differential transformer (LVDT, Model CD-375-025-010; Macrosensors, Schaevitz Technologies, Pennsauken, NJ) and signal conditioner (Model LPC-2100; Macrosensors) as previously described (15–17). Briefly, the LVDT is mounted on a chosen dorsal hand vein, which then measures and records changes in diameter of the vein by displacement of a solenoid. Subjects were studied at either 9 am or 1 pm start time based on availability, and the visit began and concluded with measurements of BP and HR. Subjects rested in the reclining position in a quiet temperature-controlled room (21–22 °C) with the ipsilateral forearm placed on a custom-constructed arm cradle that sloped upward. A heating pad was placed underneath the subject's hand to maintain skin temperature between 31–32°C throughout the study. A suitable large superficial dorsal hand vein without apparent tributaries was chosen, and a 1.91 cm (0.75 in) plastic catheter was inserted into the distal segment of that vein, downstream from the solenoid, using a 24-gauge needle. Then, 0.5% albumin (albumin [human], U.S. Pharmacopeia; Plasbumin-25; Bayer, Pharmaceutical Division, Elkhart, IN) in normal saline solution using a Harvard dual-syringe pump (Harvard Clinical Technology, South Natick, MA), starting at a rate of 12 mL/h (0.2 mL/min) was continuously infused as a “washout” period for 30 min prior to baseline measurements. Next, the ipsilateral upper arm was compressed with an automated

sphygmomanometer cuff (Rapid Inflate Straight Cuffs; Hokanson, Bellevue, WA) inflated to 45 mmHg (Rapid Cuff Inflator, model E20; Hokanson) for 2.5 min to achieve maximal venous capacitance. Three baseline measurements of the hand vein diameter were obtained, requiring the coefficient of variation (CV) to be <5%, to indicate reproducibility. This distension during albumin infusion and inflated cuff was defined as baseline, or 0% constriction.

Baseline Preconstriction and Catestatin Infusion

Human catestatin (human CHGA_{352–372}; SSMKLS-FRARAYGFRGPGPQL) was synthesized by the solid-phase method, verified by mass spectrometry, purified to ≥95% by reverse-phase (C-18) high-performance liquid chromatography (HPLC), stored as a dry powder, diluted in 0.5% albumin in normal saline, and 0.2 micron filter-sterilized prior to use. Control peptide synthesis and infusion were performed with pancreastatin.

To enable a measurable range of dilation in response to catestatin, preconstriction was first achieved in the dorsal hand vein by infusing phenylephrine, an alpha-1 selective adrenergic agonist (phenylephrine HCl, MW = 203.7 g/mole). Increasing concentrations of phenylephrine (100–7500 ng/min) were infused at 6 ml/h (0.1 ml/min) for 8 min, with responses recorded during the last 2.5 min of each dose when the cuff was inflated, until approximately ~70% constriction of the baseline hand vein diameter was achieved.

Once the goal preconstriction (~ 70%) had been reached, that particular dose of phenylephrine was continued throughout the remainder of the experiment. Three doses of catestatin were infused at 6 ml/h (0.1 ml/min) to reach target intravascular dorsal hand vein concentrations of 50, 500, and 5000 nM, which were up to ~10³ magnitude greater than what has been observed in normotensive subjects (~1.5nM) (13), assuming a typical human dorsal hand vein blood flow at ~2.5 ml/min (18). Each dose was again infused for 8 min, with responses recorded during the last 2.5 min of each dose with the sphygmomanometer cuff inflated. Throughout the study, a continuous infusion rate of 18 ml/h (0.3 ml/min) was maintained by infusion of albumin in saline at rates of 0–12 ml/h (0–0.2 ml/min) as needed.

To evaluate the stability of the phenylephrine goal dose, 11 subjects were measured again after 10 min of continuous infusion; there were no significant difference in their percent constriction from baseline ($P = 0.17$). Significance was not affected by covariates including age, sex, baseline hand vein diameter, or phenylephrine dose required.

Control CHGA Peptide

Eleven subjects (6 males and 5 females) returned on a separate day for study with a control CHGA peptide, pancreastatin (wild-type human CHGA_{273–301}-amide; PEGKGEQHSQQKEEEEEEMAVVP- QGLFRG-amide, MW = 3972 g/mol). Eight of the 11 subjects were also in the catestatin study cohort. The three subjects that participated in the control experiment alone did not differ from the catestatin subjects by age, sex, blood pressure status, baseline hand vein diameter, or dose of phenylephrine required. The pancreastatin was synthesized in the same manner as the catestatin, and verified by mass spectroscopy. The same protocol was followed as noted previously, but pancreastatin was infused in place of catestatin after phenylephrine preconstriction. Pancreastatin was infused to reach dorsal hand vein concentrations of 50 and 500 nM, which was ~10⁵ magnitude greater than that found in nondiabetic subjects (4.9 pM) (19).

Plasma Catestatin Levels and Role of Sex in Trait Determination

Six hundred twenty-two (257 males, 365 females) normotensive subjects with a mean age of 40.1 (range 15–81) years, primarily of Caucasian ancestry (86%) had plasma CHGA

precursor and catestatin levels measured. The ratio of catestatin to total CHGA, approximating processing, was determined by the formula: Processing = $100 * \frac{[\text{plasma catestatin}]}{([\text{plasma catestatin}] + [\text{plasma CHGA precursor}])}$. Plasma samples were obtained from seated subjects with a heparin-lock IV indwelling for 20 min and then promptly stored at -70°C until assay. The CHGA and catestatin radioimmunoassays were performed as previously described (14). The catestatin region (human CHGA₃₅₂₋₃₇₂) was accessed by a synthetic peptide epitope corresponding to CHGA₃₆₁₋₃₇₂. The CHGA precursor was accessed by assay using the large fragment CHGA₁₁₆₋₄₃₉. The intra- and inter-variation coefficients of variation are 3.5% and 4.7% for the CHGA₁₁₆₋₄₃₉ assay, respectively; and 4.1% and 5.9%, respectively, for the CHGA₃₆₁₋₃₇₂ assay.

Analysis and Statistics

Dorsal hand vein constriction and dilation responses were expressed as the percent reduction in vein diameter from baseline, which was during 0.5% albumin and saline infusion (maximal dilation with arm cuff inflation to 45 mmHg). The baseline was defined as the mean of 3 stable ($\pm 5\%$ CV) baseline measurements.

Results are presented as the mean value ± 1 SEM. Additive general linear models were used as association tests. Dose response curves in different groups were compared using two-way repeated measures parametric ANOVA. (SPSS 11.5 for Windows; SPSS Inc., Chicago, IL). To compare differences between groups, t- tests and univariate ANOVA were used. Correlations were determined by Pearsons correlation or nonparametric Spearman's rho. When the distribution of a trait, such as for CHGA precursor, was found to have a non-normal distribution, log transformation were performed for normalization. Geometric means are then presented. Statistical significance was set at $P < 0.05$.

The authors had full access to the data and take responsibility for their integrity. All authors have read and agree to the manuscript as written.

RESULTS

Dorsal Hand Vein Subject Characteristics

Characteristics of the hand vein study subjects are shown in Table 1. Eighteen normotensive, nonsmoking subjects were studied (11 male and 7 female) between the ages of 19–51 years (mean 33.2 years), with self-identified ethnicities of 50% Caucasian, 33% East Asian, 11% South Asian, and 6% Black. Twenty-two percent reported a positive family history of hypertension and 44% a negative, whereas it was unknown/uncertain in 33%. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) values were normal, and did not differ significantly prior to and after the study. Male and female subjects differed by their baseline weight and height, though they had no significant difference in body mass index (BMI). Male subjects also had greater baseline hand vein diameter. Figure 1 illustrates an LVDT tracing from a subject. The three baseline tracings, with CV $< 5\%$, were performed first (Figure 1A).

Dorsal Hand Vein Preconstriction by Graded Phenylephrine Infusion

Preconstriction by phenylephrine infusion achieved the goal of $\sim 70\%$ in each subject for up to nine graded doses (100–7500 ng/min). A mean phenylephrine dose of 1620 ± 460 ng/min was required in the subjects, with 37% requiring 100 ng/min and 6% each requiring 400 ng/min and 7500 ng/min. A mean hand vein pre-constriction of 69.3% (range, 44–93%) was achieved. Given a typical dorsal hand vein blood flow rate of ~ 2.5 ml/min (18) and the molecular weight, the mean phenylephrine infusion rate corresponds to an intravascular concentration of ~ 3.2 μM . The dose requirement for phenylephrine in our study subjects did

not correlate with baseline dorsal hand vein diameter, sex, or age. Male and female subjects did not have significant differences in percent hand vein constriction or the dose of phenylephrine required. Figure 1B exemplifies an LVDT tracing in a subject whose hand vein constricted to 85% with the first dose of phenylephrine (100 ng/min).

Dorsal Hand Vein Venodilation with Catestatin Graded Dose Infusion

Progressive vasodilation with increasing doses of catestatin ($F = 3.68$, $P = 0.019$) was noted. Female subjects experienced significantly more vasodilation (70.0% to 43.7%) than male subjects, as shown in Figure 2 (catestatin-by-sex, $F = 3.46$, $P = 0.024$; covariate: age). Co-variables, such as baseline hand vein diameter, ethnicity, BMI, and phenylephrine dose required, did not influence the vasodilatory response. The semi-maximal active dose (EC_{50}) of catestatin in the female subjects was estimated at ~30 nM. Figure 1C shows the LVDT tracings of a subject who experienced dose-dependent vasodilation with increasing concentrations of catestatin.

Control CHGA Peptide (Pancreastatin) Infusion

The CHGA peptide pancreastatin was infused into the dorsal hand vein of 11 subjects, as a control for the specificity of action of catestatin. With increasing concentrations of pancreastatin (0, 50, 500 nM), no significant vasodilation was noted (data not shown, $F = 0.21$, $P = 0.82$; covariate: age). Nor was an effect revealed by consideration of co-variables including sex, (pancreastatin-by-sex, with co-variate age; $F = 0.47$, $P = 0.63$), baseline hand vein diameter, BMI, or required phenylephrine dose.

Plasma Catestatin and Role of Sex in Trait Determination

In a sample of 622 normotensive subjects (365 female, 257 male) described in Table 2, males and females had significant differences in plasma catestatin and CHGA levels. Significant correlations were noted between plasma catestatin and SBP ($P = 0.007$) and plasma catestatin and sex ($P < 0.001$), whereas plasma CHGA was correlated only with sex ($P = 0.024$).

Plasma catestatin ranged from 0.03–4.4 nM, with mean values higher in females (Figure 3A, $P = 0.001$). With an upper limit of normal for circulating *endogenous* catestatin at 4.4 nM, the semi-maximal effective concentration (EC) for *exogenous* catestatin on venous tone in women (~30 nM, Figure 2) is less than one order of magnitude greater than the *endogenous* circulating concentration, suggesting that even variation of plasma catestatin near the physiologic range may influence vascular tone.

Figure 3 illustrates the differences by sex of the peptidergic traits: plasma catestatin, the CHGA precursor, and the ratio of catestatin: total CHGA. Catestatin levels are higher in females than males (by ~14%, $P = 0.001$, Figure 3A), though CHGA is lower (by ~19%, $P = 0.006$, Figure 3B). Thus, the ratio of catestatin: total CHGA, which may reflect processing of CHGA-to-catestatin is elevated by ~1.1-fold in females ($P < 0.001$, Figure 3C).

DISCUSSION

Overview

Alterations in activity of the adrenergic system have been implicated in the development of human hypertension (20). Catecholamines are co-released from their secretory vesicles with CHGA, whose bioactive peptide, catestatin, is noted to be diminished in subjects with familial predisposition for hypertension (13), suggesting that reduced catestatin levels may increase the risk of hypertension. We found that local infusion of exogenous catestatin *in vivo* resulted in vasodilation in healthy subjects, especially females (Figure 2), an effect that

was not apparent during infusion of a control CHGA peptide pancreastatin. These results are consistent with previous work in rats where catestatin reduced pressor response to activation of sympathetic outflow (6) and in *CHGA* knock-out mice (*Chga* $-/-$) that displayed elevated blood pressure, which normalized with exogenous catestatin infusion (2).

In females, vasodilation approached a plateau with the highest infusion concentration of catestatin (~ 5000 nM), a value substantially greater than physiologic levels, given that our sample of 622 normotensive subjects had a maximum catestatin level of 4.4 nM. However, substantial venodilation was noted even at the very lowest concentration of exogenous catestatin, at ~ 50 nM (Figure 2). The semi-maximal catestatin concentration (EC_{50}) for vasoactivity was ~ 30 nM (Figure 2) in the female subjects. Thus, we achieved substantial vasodilation at exogenous concentrations less than one order of magnitude greater than the prevailing endogenous range, suggesting that catestatin within the physiological range likely has functional consequences for vascular tone. We previously noted that catestatin displayed dose-dependent inhibition of nicotinic cholinergic-stimulated catecholamine secretion from chromaffin (PC12) cells with a similar EC_{50} of ~ 200 nM (21). Thus catestatin appears to be more potent in dilating the human vasculature *in vivo* than it is in blocking nicotinic secretory responses in chromaffin cells, suggesting an alternate mechanism of action.

Mechanisms of catestatin effects in hypertension and vasodilation may be multiple (5). One mechanism, previously elucidated by our lab, demonstrated that catestatin inhibits catecholamine release through noncompetitive binding at the nicotinic cholinergic receptor (21,22). Though this mechanism may contribute to both central and peripheral neuronal activity, it is unlikely to result in local vasodilation, since direct nicotinic cholinergic innervation of blood vessels has not been demonstrated. Another potential mechanism for the catestatin vasodilation is through histamine release (6,23), which may be modulated by nitric oxide (NO) (24,25). Previous studies indicate that catestatin may stimulate mast cells (23) whose histamine release may subsequently induce NOS (24,25).

Sex on Trait Determination

We have previously noted pronounced gene-by-sex interactions in genetic determination of blood pressure in the population (26) as well as marked differences in male and female responses to adrenergic stimuli in the isolated dorsal hand vein (17), with males displaying enhanced pressor/vasoconstrictor responses. Here, females had an exaggerated dilatory response to the catestatin vasodilator compared with males (Figure 2). Additionally, females also have higher endogenous plasma catestatin concentrations (by $\sim 14\%$, Figure 3), even with lower concentrations of the CHGA precursor, suggesting an enhanced conversion or processing of the CHGA precursor to the catestatin product (by ~ 1.1 -fold). Though, this ratio may only approximate processing of catestatin from a CHGA precursor and has not been validated as a marker of enzymatic conversion activity. To our knowledge, no other investigators have evaluated this measure.

It is uncertain why catestatin expression and the response to infusion is different in women, particularly when CHGA precursor levels are lower in women. In experimental animals, CHGA expression in neuroendocrine tissues was diminished in female animals compared to males due to an estrogen effect that appears to downregulate gene expression (27). Yet an increase in the proteolytic processing of CHGA to its biologically active catestatin product, similar to that determined between hypertensive and normotensive subjects (28), suggests an additional point of sex dependence. Chromogranin A is specifically cleaved to catestatin within the secretory pathway (29), and the proteases implicated in mediating this specific processing include the prohormone convertase family of serine proteases (30) and the recently described thiol protease cathepsin L (11,31). Neither sex differences in prohormone convertase expression nor potential interactions with sex hormones have been systematically

investigated. Enhanced response to catestatin infusion in women may involve lack of “saturation” by the ligand given the prevailing plasma catestatin concentrations (Figure 3) in comparison to the effective concentrations of catestatin to influence veins (Figure 2) at supra-physiologic levels. Perhaps more compelling are previous observations that sex steroids such as estrogen may magnify nitroxidergic control of the circulation in females (32–34).

The role of sex on hypertension, wherein males typically have higher BP measurements and cardiovascular morbidity and mortality than premenopausal women, is well-accepted (35). An enhanced vasodilatory capacity of the endothelium of premenopausal women has been cited to explain this gender difference (34). Estrogen, working in conjunction with catestatin, may modulate this vascular vasodilatory response (36), mediated through the NO-cyclic guanosine monophosphate (cGMP) pathway. Nitric oxide is a powerful vasodilator *in vivo* whose actions are often the result of stimulation of the soluble guanylate cyclase which activates the second messenger cGMP (37). Estrogen enhances the bioavailability of NO through increased NOS expression (33). As previously noted, studies have indicated that catestatin may stimulate mast cells (23) whose histamine release may subsequently induce NO (24). Further research is needed to determine if estrogen and catestatin may work together or synergistically to increase NO in female subjects.

Limitations and Advantages of This Study

The hand vein system—This study utilized the superficial dorsal hand vein technique (38) to isolate a vascular bed in order to avoid systemic baroreceptor/counterregulatory responses in the evaluation of vascular reactivity to exogenous catestatin infusion. Indeed, no changes in BP or HR were noted post-phenylephrine/catestatin infusion (Table 1). The venous system is desirable to study due to its accessibility, low intravascular pressure, thin walls, and distensibility (16). Veins do not experience the elevated hydrostatic pressure of the arterial tree, and hence interindividual differences in venous responses cannot be ascribed to the consequences of systemic hypertension. The sensitivity and innervation of arterial and a venous β -adrenergic receptor may differ, and thus our results may not be readily extrapolated to other vascular beds *in vivo* (39). Further studies of catestatin in an arterial bed may be warranted; indeed, we previously found that catestatin infusion into the human brachial artery did increase forearm blood flow (19).

Pancreastatin infusion (negative control)—This negative control was performed to demonstrate catestatin’s specificity of action. Given the predominant action of pancreastatin on glycemic control (19), in both carbohydrate and lipid metabolism (40), it is not surprising that it did not affect vascular responses in this study. Pancreastatin levels have been noted to be increased in hypertensive subjects though (41) and correlate with norepinephrine levels (42), but its actions may relate to the metabolic syndrome and to insulin-resistance. A previous study of pancreastatin in humans evaluated forearm glucose uptake during direct brachial arterial infusion; catestatin was used as a control and had no metabolic effects (19).

Pharmacologic interactions—Our findings could potentially be confounded by pharmacologic interactions between catestatin and adrenergic receptors in the vasculature, given our use of an α -1 selective adrenergic agonist for precontraction of the hand vein. In animal studies, cardiotropic effects of catestatin were abolished by inhibition of beta adrenergic receptors (7,8). Further investigation is needed to determine whether there may be cross-talk between catestatin and adrenergic receptors and/or signaling.

Healthy subjects—This study probed a local vascular bed of healthy, non-smoking, and normotensive subjects. Further study of hypertensive subjects may elucidate whether

catestatin levels decline, especially in women or the elderly, as a potential contributor to their higher prevalence of systolic hypertension. Clinically, catestatin concentrations might be assessed for possible pharmacologic profiling or as vasodilator treatment in hypertensive subjects with decreased basal levels, as has been accomplished in animal studies (2).

A previous study indicated that plasma catestatin varies based on the family history of hypertension (13). Here we found no significant differences in vasoreactivity to catestatin when stratifying subjects by family history of hypertension, but we likely did not have adequate statistical power in this study of only 18 subjects, of whom one-third had an indeterminate/uncertain family history of hypertension. Similarly, the small number of subjects in each ethnic group may not have had sufficient power to detect subtle differences between the groups, either as co-variables or as dominant effects.

CONCLUSIONS

Our results establish dose-dependent venodilation with *in-vivo* infusion of the CHGA bioactive peptide catestatin in a local vascular bed of healthy subjects, which reinforces previous evidence that catestatin may contribute to endogenous vascular tone (13). Female subjects exhibited greater vasodilation and had higher plasma levels of catestatin with an increased processing of CHGA. Additional studies may be required to elucidate how catestatin and sex interact to influence vascular tone and hypertension, including its molecular mechanism of action, and also to determine whether there may be clinical utility in establishing catestatin levels that predict the risk of hypertension. Finally, the results suggest that catestatin (or its derivatives) might provide new targets for pharmacologic dilation in vascular disease states such as hypertension.

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References

1. Kim T, Tao-Cheng JH, Eiden LE, Loh YP. Chromogranin A, an “on/off” switch controlling dense-core secretory granule biogenesis. *Cell*. 2001; 106:499–509. [PubMed: 11525735]
2. Mahapatra NR, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Mahata SK. Hypertension from targeted ablation of chromogranin A can be rescued by the human ortholog. *J Clin Invest*. 2005; 115:1942–1952. [PubMed: 16007257]
3. Takiyyuddin MA, Parmer RJ, Kailasam MT, et al. Chromogranin A in human hypertension. Influence of heredity. *Hypertension*. 1995; 26:213–220. [PubMed: 7607727]
4. Taupenot L, Harper KL, O'Connor DT. The chromogranin-secretogranin family. *N Engl J Med*. 2003; 348:1134–1149. [PubMed: 12646671]
5. Mahapatra NR. Catestatin is a novel endogenous peptide that regulates cardiac function and blood pressure. *Cardiovasc Res*. 2008; 80:330–338. [PubMed: 18541522]
6. Kennedy BP, Mahata SK, O'Connor DT, Ziegler MG. Mechanism of cardiovascular actions of the chromogranin A fragment catestatin in vivo. *Peptides*. 1998; 19:1241–1248. [PubMed: 9786174]
7. Mazza R, Gattuso A, Mannarino C, Brar BK, Barbieri SF, Tota B, Mahata SK. Catestatin (chromogranin A344-364) is a novel cardiosuppressive agent: inhibition of isoproterenol and endothelin signaling in the frog heart. *Am J Physiol Heart Circ Physiol*. 2008; 295:H113–122. [PubMed: 18469147]

8. Angelone T, Quintieri AM, Brar BK, Limchaiyawat PT, Tota B, Mahata SK, Cerra MC. The antihypertensive chromogranin a peptide catestatin acts as a novel endocrine/paracrine modulator of cardiac inotropism and lusitropism. *Endocrinology*. 2008; 149:4780–4793. [PubMed: 18535098]
9. Metz-Boutigue MH, Garcia-Sablone P, Hogue-Angeletti R, Aunis D. Intracellular and extracellular processing of chromogranin A. Determination of cleavage sites. *Eur J Biochem*. 1993; 217:247–257. [PubMed: 8223562]
10. Simon JP, Bader MF, Aunis D. Secretion from chromaffin cells is controlled by chromogranin A-derived peptides. *Proc Natl Acad Sci U S A*. 1988; 85:1712–1716. [PubMed: 3422758]
11. Yasothornsrikul S, Greenbaum D, Medzihradsky KF, Toneff T, Bunday R, Miller R, Schilling B, Petermann I, Dehnert J, Logvinova A, Goldsmith P, Neveu JM, Lane WS, Gibson B, Reinheckel T, Peters C, Bogoy M, Hook V. Cathepsin L in secretory vesicles functions as a prohormone-processing enzyme for production of the enkephalin peptide neurotransmitter. *Proc Natl Acad Sci U S A*. 2003; 100:9590–9595. [PubMed: 12869695]
12. Parmer RJ, Mahata M, Gong Y, Mahata SK, Jiang Q, O'Connor DT, Xi XP, Miles LA. Processing of chromogranin A by plasmin provides a novel mechanism for regulating catecholamine secretion. *J Clin Invest*. 2000; 106:907–915. [PubMed: 11018079]
13. O'Connor DT, Kailasam MT, Kennedy BP, Ziegler MG, Yanaihara N, Parmer RJ. Early decline in the catecholamine release-inhibitory peptide catestatin in humans at genetic risk of hypertension. *J Hypertens*. 2002; 20:1335–1345. [PubMed: 12131530]
14. Rao F, Wen G, Gayen JR, Das M, Vaingankar SM, Rana BK, Mahata M, Kennedy BP, Salem RM, Stridsberg M, Abel K, Smith DW, Eskin E, Schork NJ, Hamilton BA, Ziegler MG, Mahata SK, O'Connor DT. Catecholamine release-inhibitory peptide catestatin (chromogranin A(352–372)): naturally occurring amino acid variant Gly364Ser causes profound changes in human autonomic activity and alters risk for hypertension. *Circulation*. 2007; 115:2271–2281. [PubMed: 17438154]
15. Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins–2. *Br J Clin Pharmacol*. 1994; 38:289–305. [PubMed: 7833218]
16. Aellig WH. Clinical pharmacology, physiology and pathophysiology of superficial veins–1. *Br J Clin Pharmacol*. 1994; 38:181–196. [PubMed: 7826819]
17. King D, Etzel JP, Chopra S, Smith J, Cadman PE, Rao F, Funk SD, Rana BK, Schork NJ, Insel PA, O'Connor DT. Human response to alpha2-adrenergic agonist stimulation studied in an isolated vascular bed in vivo: Biphasic influence of dose, age, gender, and receptor genotype. *Clin Pharmacol Ther*. 2005; 77:388–403. [PubMed: 15900285]
18. Chalon S, Moreno H Jr, Benowitz NL, Hoffman BB, Blaschke TF. Nicotine impairs endothelium-dependent dilatation in human veins in vivo. *Clin Pharmacol Ther*. 2000; 67:391–397. [PubMed: 10801248]
19. O'Connor DT, Cadman PE, Smiley C, Salem RM, Rao F, Smith J, Funk SD, Mahata SK, Mahata M, Wen G, Taupenot L, Gonzalez-Yanes C, Harper KL, Henry RR, Sanchez-Margalet V. Pancreastatin: multiple actions on human intermediary metabolism in vivo, variation in disease, and naturally occurring functional genetic polymorphism. *J Clin Endocrinol Metab*. 2005; 90:5414–5425. [PubMed: 15956083]
20. Goldstein DS. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension*. 1983; 5:86–99. [PubMed: 6336721]
21. Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, Gill BM, Parmer RJ. Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. *J Clin Invest*. 1997; 100:1623–1633. [PubMed: 9294131]
22. Mahata SK, Mahata M, Parmer RJ, O'Connor DT. Desensitization of catecholamine release. The novel catecholamine release-inhibitory peptide catestatin (chromogranin a344–364) acts at the receptor to prevent nicotinic cholinergic tolerance. *J Biol Chem*. 1999; 274:2920–2928. [PubMed: 9915830]
23. Kruger PG, Mahata SK, Helle KB. Catestatin (CgA344–364) stimulates rat mast cell release of histamine in a manner comparable to mastoparan and other cationic charged neuropeptides. *Regul Pept*. 2003; 114:29–35. [PubMed: 12763637]

24. Li H, Burkhardt C, Heinrich UR, Brausch I, Xia N, Forstermann U. Histamine upregulates gene expression of endothelial nitric oxide synthase in human vascular endothelial cells. *Circulation*. 2003; 107:2348–2354. [PubMed: 12707234]
25. Tanimoto A, Wang KY, Murata Y, Kimura S, Nomaguchi M, Nakata S, Tsutsui M, Sasaguri Y. Histamine upregulates the expression of inducible nitric oxide synthase in human intimal smooth muscle cells via histamine H1 receptor and NF-kappaB signaling pathway. *Arterioscler Thromb Vasc Biol*. 2007; 27:1556–1561. [PubMed: 17478759]
26. Rana BK, Insel PA, Payne SH, Abel K, Beutler E, Ziegler MG, Schork NJ, O'Connor DT. Population-based sample reveals gene-gender interactions in blood pressure in White Americans. *Hypertension*. 2007; 49:96–106. [PubMed: 17159089]
27. Fischer-Colbrie R, Schmid K, Mahata SK, Laslop A, Bauer J. Sex-related differences in Chromogranin A, Chromogranin B, and Secretogranin II Gene Expression in Rat Pituitary. *Journal of Neuroendocrinology*. 1992; 4:125–130. [PubMed: 21554587]
28. O'Connor DT, Zhu G, Rao F, Taupenot L, Fung MM, Das M, Mahata SK, Mahata M, Wang L, Zhang K, Greenwood TA, Shih PA, Cockburn MG, Ziegler MG, Stridsberg M, Martin NG, Whitfield JB. Heritability and genome-wide linkage in US and Australian twins identify novel genomic regions controlling chromogranin a: implications for secretion and blood pressure. *Circulation*. 2008; 118:247–257. [PubMed: 18591442]
29. Taylor CV, Taupenot L, Mahata SK, Mahata M, Wu H, Yasothornsrikul S, Toneff T, Caporale C, Jiang Q, Parmer RJ, Hook VY, O'Connor DT. Formation of the catecholamine release-inhibitory peptide catestatin from chromogranin A. Determination of proteolytic cleavage sites in hormone storage granules. *J Biol Chem*. 2000; 275:22905–22915. [PubMed: 10781584]
30. Eskeland NL, Zhou A, Dinh TQ, Wu H, Parmer RJ, Mains RE, O'Connor DT. Chromogranin A processing and secretion: specific role of endogenous and exogenous prohormone convertases in the regulated secretory pathway. *J Clin Invest*. 1996; 98:148–156. [PubMed: 8690787]
31. Lee JC, Taylor CV, Gaucher SP, Toneff T, Taupenot L, Yasothornsrikul S, Mahata SK, Sei C, Parmer RJ, Neveu JM, Lane WS, Gibson BW, O'Connor DT, Hook VY. Primary sequence characterization of catestatin intermediates and peptides defines proteolytic cleavage sites utilized for converting chromogranin a into active catestatin secreted from neuroendocrine chromaffin cells. *Biochemistry*. 2003; 42:6938–6946. [PubMed: 12795588]
32. Case J, Davison CA. Estrogen alters relative contributions of nitric oxide and cyclooxygenase products to endothelium-dependent vasodilation. *J Pharmacol Exp Ther*. 1999; 291:524–530. [PubMed: 10525067]
33. Mendelsohn ME, Karas RH. Molecular and cellular basis of cardiovascular gender differences. *Science*. 2005; 308:1583–1587. [PubMed: 15947175]
34. Kublickiene K, Luksha L. Gender and the endothelium. *Pharmacol Rep*. 2008; 60:49–60. [PubMed: 18276985]
35. Burt VL, Whelton P, Roccella EJ, Brown C, Cutler JA, Higgins M, Horan MJ, Labarthe D. Prevalence of hypertension in the US adult population. Results from the Third National Health and Nutrition Examination Survey, 1988–1991. *Hypertension*. 1995; 25:305–313. [PubMed: 7875754]
36. Leinwand LA. Sex is a potent modifier of the cardiovascular system. *J Clin Invest*. 2003; 112:302–307. [PubMed: 12897194]
37. Knowles RG, Palacios M, Palmer RM, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. *Proc Natl Acad Sci U S A*. 1989; 86:5159–5162. [PubMed: 2567995]
38. Aellig WH. A new technique for recording compliance of human hand veins. *Br J Clin Pharmacol*. 1981; 11:237–243. [PubMed: 7213525]
39. Stein CM, Deegan R, Wood AJ. Lack of correlation between arterial and venous beta-adrenergic receptor sensitivity. *Hypertension*. 1997; 29:1273–1277. [PubMed: 9180628]
40. Zhang K, Rao F, Wen G, Salem RM, Vaingankar S, Mahata M, Mahapatra NR, Lillie EO, Cadman PE, Friese RS, Hamilton BA, Hook VY, Mahata SK, Taupenot L, O'Connor DT. Catecholamine storage vesicles and the metabolic syndrome: The role of the chromogranin A fragment pancreastatin. *Diabetes Obes Metab*. 2006; 8:621–633. [PubMed: 17026486]

41. Sanchez-Margalet V, Valle M, Lobon JA, Maldonado A, Escobar-Jimenez F, Olivan J, Perez-Cano R, Goberna R. Increased plasma pancreastatin-like immunoreactivity levels in non-obese patients with essential hypertension. *J Hypertens*. 1995; 13:251–258. [PubMed: 7615956]
42. Sanchez-Margalet V, Valle M, Lobon JA, Escobar-Jimenez F, Perez-Cano R, Goberna R. Plasma pancreastatin-like immunoreactivity correlates with plasma norepinephrine levels in essential hypertension. *Neuropeptides*. 1995; 29:97–101. [PubMed: 7477767]

Figure 1.

Example of dorsal hand vein tracings from the linear variable differential transformer (LVDT). All hand vein diameter measurements are taken at maximal venous capacitance, at peak height after inflation of the sphygmomometer to 45 mmHg. (A) Baseline. Three tracings are performed with 0.5% albumin/saline infusion. The baseline measurements must have a coefficient of variance <5% for reproducibility. (B) Phenylephrine precontraction. Five minutes of infusion is performed before the cuff is inflated to reach maximal venous capacitance in the final ~2.5 min of the infusion and measurement is taken after plateau is reached. The goal is to achieve ~70% constriction from baseline. In this example, 85% constriction from baseline was reached with the first dose of phenylephrine (100 ng/ min). (C) Catestatin. Three doses of catestatin are infused to reach dorsal hand vein concentration of ~50, ~500, ~5000 nM. As noted above, ~5 min of infusion is performed before the cuff is inflated to reach maximal venous capacitance in the final ~2.5 minutes of the infusion and measurement is taken after plateau is reached. This subject experienced mild dose-dependent vasodilation, noted by the increase in peak height with each concentration of catestatin.

Figure 2.

Exogenous catestatin infusion into the dorsal hand vein: Stratification by sex. Catestatin exhibited dose-dependent vasodilation ($P = 0.019$), with the effect most prominent in female subjects ($P = 0.024$; covariate: age). Assuming maximal venodilation with the highest concentration of catestatin, the EC_{50} (semi-maximal effective concentration) for females was ~ 30 nM.

Figure 3.

Endogenous catestatin formation, circulation, and catecholamines: Role of sex in trait determination. Results are presented from 622 ambulatory normotensive subjects. (A) Plasma catestatin (epitope: CHGA₃₆₁₋₃₇₁). Females had higher plasma catestatin levels ($P = 0.001$). Range of catestatin: 0.03 to 4.4 nM. (B) Plasma CHGA precursor (epitope: CHGA₁₁₆₋₄₃₉). Females had lower CHGA precursor ($P = 0.006$). (C) Catestatin to total CHGA ratio. This percent processing was estimated as: $100 \cdot [\text{plasma catestatin}] / ([\text{plasma catestatin}] + [\text{plasma CHGA precursor}])$. Females exhibited increased CHGA processing to catestatin ($P < 0.001$).

Table 1

Characteristics of hand vein subjects: Demographic, physical, and physiological. The left side of the table is for all subjects together. No differences were noted between pre- and post-study vital signs ($P > 0.05$). The right side shows the male and female subjects separately with standard errors. The P value reflects differences between the sexes

Baseline characteristics	Mean N = 18	SEM	Males N = 11	Females N = 7	P Value
Age (years)	33.2	2.8	30.5 ± 3.6	37.4 ± 4.6	0.25
Weight (kg)	74.3	3.8	81.8 ± 4.5	62.6 ± 3.8	0.008
Height (m)	1.7	0.03	1.77 ± 0.02	1.61 ± 0.02	<0.001
BMI (kg/m ²)	25.1	0.86	25.8 ± 1.1	24.1 ± 1.4	0.35
Baseline dorsal hand vein diameter (mm)	0.73	0.07	0.84 ± 0.1	0.55 ± 0.03	0.043
Dose of phenylephrine required for vasoconstriction to ~70% (ng/min)	1620	460	1900 ± 700	1171 ± 470	0.46
Hand vein constriction with phenylephrine (%)	69.3	3.4	68.6 ± 5.0	70.3 ± 4.3	0.82
Dose phenylephrine/percent hand vein constriction (ng/min/percent constriction)	21.8	6.2	26.0 ± 9.4	15.3 ± 6.0	0.42
	%		%	%	
Ethnicity					0.81
White	50		54.5	42.9	
Black	5.6		0	14.3	
East Asian	33.3		36.4	28.6	
South Asian	11.1		9.1	14.3	
Family history of hypertension					0.011
Yes	22.2		0	57.1	
No	44.4		54.5	28.6	
Unknown/indeterminate	33.3		45.5	14.3	
Non-smokers	100		100	100	
Prestudy vital signs		SEM			
Systolic blood pressure (mmHg)	121.4	2.3	123.8 ± 3.7	115.4 ± 5.5	0.21
Diastolic blood pressure (mmHg)	76.1	1.6	72.1 ± 3.2	71.9 ± 4.7	0.97
Pulse (beats/minute)	72.1	1.9	67.6 ± 2.2	72.7 ± 4.8	0.30
Post-study vital signs		SEM			
Systolic blood pressure (mmHg)	122.4	2.8	122.7 ± 2.2	117.4 ± 6.1	0.37
Diastolic blood pressure (mmHg)	78.5	1.6	77.6 ± 2.4	76.1 ± 3.9	0.74
Pulse (beats/minute)	72.1	1.8	68.6 ± 0.15	72.0 ± 2.4	0.39

Subject characteristics for plasma chromogranin A (CHGA), catestatin, and catestatin: CHGA ratio. Means and standard errors are reported. Demographic information is also noted for all subjects together on the left side, and males and female subjects separately on the right side, with P values noting differences between the sexes. Catestatin: CHGA ratio = $100 \cdot [\text{plasma catestatin}] / ([\text{plasma catestatin}] + [\text{plasma CHGA precursor}])$

Table 2

Subject demographics (N = 622)	Mean	SEM	Males N = 257	Females N = 365	P value
Age (years)	40.1	0.58	40.4 ± 0.83	39.8 ± 0.79	>0.05
Weight (kg)	74	0.74	84.9 ± 1.08	66.4 ± 0.79	<0.001
Height (m)	1.7	0.004	1.78 ± 0.0043	1.64 ± 0.0039	<0.001
BMI (kg/m ²)	25.6	0.22	26.8 ± 0.33	24.8 ± 0.29	<0.001
SBP (mmHg)	129.1	0.69	132.5 ± 1.1	127.9 ± 0.84	0.001
DBP (mmHg)	70.1	0.44	71.0 ± 1.0	69.9 ± 0.47	0.31
Chromogranin A (CHGA ₁₁₆₋₄₃₉), nM	4.20	0.16	4.65 ± 0.33	3.89 ± 0.15	0.006*
Catestatin (CHGA ₃₅₂₋₃₇₂), nM	1.25	0.22	1.14 ± 0.027	1.30 ± 0.033	0.001*
Catestatin: CHGA ratio, %	25.0	0.44	23.1 ± 0.0064	26.3 ± 0.0058	<0.001*
Percentages					
Sex	41.3% Male		58.7% Female		
Ethnicity (self-reported)	86.0% Caucasian		12.4% African-American		1.6% Other
Family history of hypertension	42.9% Yes		49.5% No		7.2% Unknown
Blood pressure status	100% Normotensive				

* See Figure 3 for graphs of these differences by sex.