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Review

Catestatin is a novel endogenous peptide that regulates cardiac function and blood pressure

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Catestatin is a 21-amino acid residue, cationic and hydrophobic peptide that is formed endogenously by proteolytic cleavage of its precursor chromogranin A, a major protein co-stored and co-released with catecholamines from the storage vesicles in adrenal chromaffin cells and adrenergic neurons. This peptide exhibits potent catecholamine release-inhibitory activity by acting on the neuronal nicotinic acetylcholine receptor. It also stimulates histamine release from mast cells *via* heterotrimeric G-proteins in a receptor-independent manner. Plasma levels of catestatin are diminished not only in hypertensive patients but also in their still-normotensive offspring, indicating its role in the pathogenesis of hypertension. Consistently, exogenous catestatin rescues hypertension in chromogranin A knockout mice and diminishes blood pressure responses to activation of sympathetic outflow in rats. These hypotensive actions of catestatin may be caused directly by autocrine inhibition of catecholamine release from the sympathoadrenal system and indirectly by paracrine stimulation of the potent vasodilator histamine release from mast cells. Recently, three human variants of catestatin displaying differential potencies for inhibition of catecholamine secretion have been identified. One of these variants (Gly364Ser) causes increased baroreceptor sensitivity, increased cardiac parasympathetic activity, and decreased cardiac sympathetic activity, and it seems to alter the risk for hypertension. These cardiovascular effects may have resulted by action of this peptide in the baroreceptor centre of the nucleus tractus solitarius. Thus, accumulating evidence documents the endogenous peptide catestatin as a novel regulator of cardiac function and blood pressure.

1. Introduction

The sympathoadrenal system consisting of sympathetic postganglionic neurons and adrenal medullary chromafin cells release the catecholamines epinephrine, norepinephrine, and dopamine into the blood stream upon activation of the neuronal nicotinic receptors by acetylcholine in preparation for the 'fight or flight' reactions.[1–4] The predominant catecholamine released from the adrenal chromaffin cells is epinephrine, while that from the sympathetic postganglionic neurons is norepinephrine.[1] Norepinephrine produces increased vascular resistance and increased systolic blood pressure (SBP) as well as diastolic blood pressure (DBP); epinephrine results in increased heart rate and cardiac output with increased SBP but decreased DBP.[1] Dopamine also plays an important role in the pathogenesis of hypertension by regulating epithelial sodium transport, vascular smooth muscle contractility, production of reactive oxygen species as well as by interacting with the renin–angiotensin and sympathetic nervous systems.[5] Indeed, increased sympathoadrenal tone including elevated levels of secreted catecholamines results in hypertension and can lead to serious cardiovascular complications.[1–3,6–8]

Catcholamines are co-stored and co-released with a group of acidic secretory proteins [chromogranins, the index member being chromogranin A (CHGA)], ATP, peptides, acetylcholine, and calcium from the storage vesicles in adrenal chromaffin cells and adrenergic neurons.[9–11] CHGA may be involved in the process of formation of these vesicles by virtue of its pH, calcium, and catecholamine-dependent aggregation properties.[12] Indeed, recent *in vitro* as well as *in vivo* studies demonstrated crucial role of CHGA for biogenesis of chromaffin granules.[13–15]

Human *CHGA* gene consists of eight exons separated by seven introns and has been mapped into chromosome 14q32.[16] It translates to a 457 amino acids protein containing a signal peptide of 18 amino acids (*Figure 1*). The mature human CHGA protein (439 amino acids) has many pairs of basic residues that are potential cleavage sites for prohormone processing endoproteases (such as prohormone convertases PC1/3 and PC2), which also coexist in the secretory granules.[9,17] Indeed, several cleavage products of CHGA have been identified to date and have been shown to exhibit important biological functions. For example,

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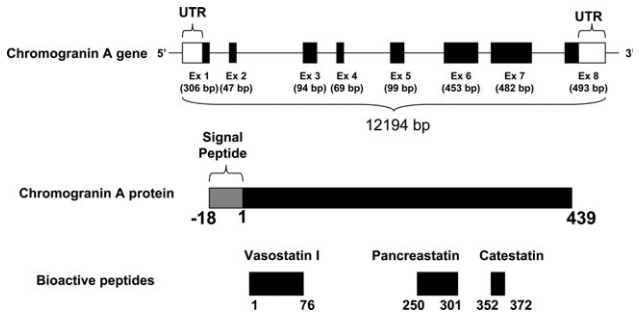

Figure 1 Schematic representation of human chromogranin A gene, protein, and some of its biologically active peptides. Human chromogranin A gene spans 12 194 bp in chromosome 14q32 and consists of eight exons giving rise to a 2043 nucleotide transcript, of which 1374 nucleotide is processed for translation. UTR, untranslated region. Chromogranin A protein consists of 457 amino acid residues, which matures to a 439 amino acid residues protein after removal of the signal peptide. The mature protein undergoes proteolytic cleavages to produce several biologically active peptides. Only those bioactive peptides that are discussed in this article are included in this figure. The positions of the amino acid residues in the peptides are numbered with respect to their positions in the matured protein. The exon/intron structure and the protein products are not drawn to scale.

vasostatin (human $CHGA_{1-76}$) acts as a vasodilator; pancreastatin (human $CHGA_{250-301}$) is a dysglycemic hormone, and catestatin (human $CHGA_{352-372}$) exhibits catecholamine release-inhibitory function[9,17] (*Figure 1*).

Catestatin (human sequence: SSMKLSFRARAYGFRGPGPQL) is a cationic and hydrophobic peptide.[18] Although the proteolytic enzymes involved for processing of this peptide from CHGA are yet to be identified, the serine protease plasmin and cysteine protease Cathepsin L are the likely candidates.[19–22] Recent studies have documented that lower plasma level of catestatin is a risk factor for development of hypertension in humans,[23] that a naturally occurring human variant of catestatin alters autonomic function and blood pressure,[24] and that arterial hypertension of *chga* knockout mouse is rescued by exogenous injection of catestatin.[15] Thus, catestain appears to play crucial roles in regulating the cardiovascular functions. These important functions of this novel peptide and the possible underlying mechanisms will be reviewed in this article.

2. Catestatin acts as a potent catecholamine release inhibitory peptide: *in vitro* studies

2.1 History of discovery

Secretory function of the parent cells was reported to be affected by several CHGA-derived peptides such as pancreastatin, originally isolated from porcine pancreas, which inhibits glucose-stimulated insulin release from islet β cells,[25] and vasostatin, which inhibits parathyroid hormone release and relaxes vascular smooth muscles.[26] Thus, there was a notion that CHGA fragments may inhibit release of further hormones. Interestingly, fragmentation of bovine CHGA generated peptide(s) that inhibited acetylcholine-evoked catecholamine secretion from cultured adrenal medullary chromaffin cells.[27] Later on, a systematic approach was undertaken to identify the specific peptide(s) responsible for such function. Fifteen peptides (19–25 amino acids residues in length) spanning ~80% of the length of mature bovine CHGA were synthesized and tested for their efficacies to block nicotine-induced catecholamine secretion from rat pheochromocytoma PC12 cells.[28] Among these various peptides, only bovine $CHGA_{344-364}$ (RSMRLSFRARGYGFRGPGLQL) displayed profound effect blocking over 90% of the nicotine-stimulated catecholamine secretion. This 21-amino acid peptide was named 'catestatin' because of its high efficacy and potency (IC_{50} ~200–300 nM) to inhibit catecholamine release.[28,29]

2.2 Active core region and crucial amino acid residues in the bovine catestatin

To identify the minimal active region within bovine catestatin ($CHGA_{344-364}$), potencies of synthetic serially deleted (N-terminal, C-terminal, or bidirectional) mutants were tested to inhibit nicotine-evoked catecholamine secretion from PC12 cells and it was found that the N-terminal 15-amino acid residue fragment $CHGA_{344-358}$ constituted the completely active core peptide.[29] Within this active core (that was sufficient to exert catestatin's nicotinic cholinergic-stimulated secretion inhibitory effect), the amino acid residues Met346, Leu348, Phe350, Arg351, Arg353, Gly354, Tyr355, Phe357, and Arg358 appeared to play significant roles as revealed by selective alanine substitution mutagenesis.[29] Among these nine amino acids, the arginine residues Arg351, Arg353, and Arg358 may be of greater importance because substitution of any of these by alanine in the 21-amino acids peptide caused profound (~4–5.5-fold) loss of potency.[18] Consistently, molecular modelling studies suggested the presence of an electropositive Arg-rich loop (R351AR353GYGFR358) that may serve as a crucial domain in the bovine catestatin for its binding at the nAChR.[18]

2.3 Physiological relevance

Although the concentration of catestatin in the vicinity of bovine chromaffin cells under physiological conditions is not known, the CHGA concentration in bovine serum has been reported to be ~7 nM.[30] Of note, the concentration of CHGA in the bovine chromaffin granules is considered to be ~4 mM[31] and that in the local extracellular space of the exocytotic pore just after exocytosis is thought to be ~0.4 mM.[28] Therefore, an IC_{50} of ~200–300 nM for bovine catestatin to inhibit catecholamine secretion may be physiologically relevant.

2.4 Activity of the bovine catestatin and its human ortholog across catecholaminergic cell types

Bovine catestatin also effectively blocked nicotine/acetylcholine stimulated catecholamine secretion from primary cultures of bovine adrenal chromaffin cells,[28] nerve growth factor-differentiated PC12 cells,[28] and mouse chromaffin cells.[32] Consistent with these observations, the human ortholog (which has ~80% sequence homology with bovine catestatin; *Figure 2*) was found to be similarly potent (with an IC_{50} ~500–800 nM) and effective in inhibiting nicotine-evoked catecholamine secretion from PC12 cells.[28,33,34] The human catestatin also inhibited nicotinic cholinergic stimulated catecholamine release from primary cultures of mouse hippocampal neurons.[24] Thus, catestatin displayed its activity across species (consistent with the sequence conservation among mammals; *Figure 2*) as well as on catecholaminergic cells of various origins.

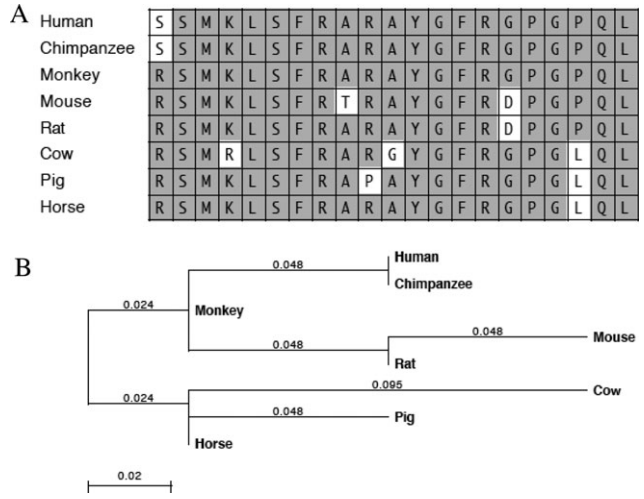

Figure 2 Clustal W analysis of the catestatin sequences across mammalian species. (*A*) Alignment of catestatin peptide region in eight mammals. The UNIPROT accession numbers of the sequences used for this analysis are: human, Q86T07; monkey, Q4R4V1; rat, Q9R1B7; horse, Q9XS63; mouse, P26339; pig, P04404; cow, P05059. The chimpanzee sequence was obtained from Wen *et al*.[44] A high degree of conservation of the sequence is evident. (*B*) Phylogenetic tree of the catestatin sequences. The multiple sequence alignment result was used to generate the phylogenetic tree, which shows the relative distances of the catestatin peptides among the mammals.

2.5 High potency and non-competitive antagonism to nicotinic cholinergic stimulation

Comparison of catestatin's relative potency with respect to the well-studied catecholamine release inhibitory peptide substance P (RPKPQQFFGLM) demonstrated its consistently superior potency; at the highest nicotine dose (1000 μM), catestatin was ∼16-fold more potent than substance P.[28] Of note, the antagonistic action of catestatin is non-competitive in nature because upon stimulation of PC12 cells with a spectrum of doses of nicotine, alone or with catestatin, nicotine could not overcome catestatin's inhibitory effect at any dose.[28] It was also observed that catestatin inhibited catecholamine secretion more potently and effectively at all nicotine doses when compared with hexamethonium, a classical non-competitive neuronal nicotinic cholinergic antagonist.[28] These findings documented catestatin as a potent, non-competitive neuronal nicotinic acetylcholine receptor (nAChR) antagonist.

2.6 Further evidence and mechanism of the antagonism of catestatin to nicotinic cholinergic receptor

To investigate the specificity of catestatin to the nAChR, the physiological trigger for catecholamine release, experiments were performed to test whether it can block catecholamine release from PC12 cells triggered by several other chromaffin cell secretagogues that act at stages in the pathway later than the nAChR, including membrane depolarization (55 mM KCl) to open voltage-gated calcium channels, an alkaline earth (2 mM $BaCl_2$) to block cell surface K^+ channels and thereby depolarize the cell membrane, a calcium ionophore (1 mM A23187) to admit extracellular Ca^{2+} to the cytosol, or alkalinization (1 mM chloroquine) of the secretory vesicles.[28,33,34] Additionally, we tested whether catestatin can modulate catecholamine release stimulated by 100 mM ATP acting on the P_{2x} purinergic receptor, or by 200 nM pituitary adenylyl cyclase activating polypeptide (PACAP) acting on PACAP type 1 (PAC1) G-protein coupled receptor thus functioning at different classes of receptors from the nAChR.[28,33,34] It was found that both the bovine and human catestatins suppressed catecholamine release only when triggered by nicotine and not when secretion was caused by agents acting at later stages (distal to the nAChR) in the secretory pathway, nor by agents acting on other receptor classes.[28,33,34] Consistent with these observations, catestatin also blocked catecholamine release from PC12 cells evoked by two other nicotinic cholinergic agonists, anatoxin and epibatidine.[33] Of note, no effect of pertussis toxin was observed on the nicotine-induced catecholamine release inhibitory effect of bovine catestatin indicating lack of involvement of the inhibitory G-proteins.[28] These observations clearly suggest catestatin's specificity for the physiological nAChR.

To confirm that the catecholamine release inhibitory effect of catestatin is indeed mediated by nAChR, electrophysiological experiments (that provide a better time resolution compared to the above-mentioned catecholamine secretion assays in which the cells are stimulated for several minutes) studying the inward currents elicited by acetylcholine in voltage-clamped *Xenopus laevis* oocytes expressing different combinations of nAChR subunits were carried out.[32] These experiments were also crucial to determine which subunit combination of the pentameric forms of the nAChR are preferentially blocked by catestatin.[32] Catestatin potently blocked all the subtypes studied with IC_{50} (catestatin concentration eliciting 50% blockade of maximal current) values of 0.3 μM for α7, 0.4 μM for both α3β2 and α3β4, and 1.7 μM for α4β2 receptors. Interestingly, in the case of α3β4 subtype, the main class of nAChRs on adrenal chromaffin cells,[4,35,36] catestatin inhibited the inward current in a reversible, non-competitive, voltage- and use-dependent manner, suggesting the open state of the channel as a target.[32] This observation is consistent with its non-competitive blockade of nicotine-evoked catecholamine release from chromaffin cells.[28] Thus, catestatin appears to play an autocrine regulatory role in exocytotic release of catecholamines through its interaction with different native nAChR subtypes while the extent of receptor blockade by the peptide may be acutely regulated by the intensity and duration of the presynaptic stimulus.[32]

The nicotinic cholinergic signal transduction involves binding of nicotine or the physiologic secretagogue acetylcholine to the agonist pocket on the nAChR, triggering Na^+ influx and consequent membrane depolarization, which activates Ca^{2+} influx through voltage-gated channels and leads to exocytotic release of the granule cargo. Bovine catestatin blocked nicotine-stimulated uptake of $^{22}Na^+$ into PC12 cells in a dose-dependent manner with an IC_{50} ∼250 nM, which is very much similar to the IC_{50} for nicotine-stimulated catecholamine release inhibition.[28] Corroboratively, the human catestatin inhibited $^{22}Na^+$ translocation into PC12 cells by ∼90%, which was similar to its extent of catecholamine release inhibition.[33] Consistent with these findings, bovine catestatin completely abolished the nicotine-evoked $^{45}Ca^{2+}$ uptake into PC12 cells.[28] It should also be noted that in mouse chromaffin cells, catestatin significantly reduced the acetylcholine-stimulated increase of cytosolic Ca^{2+} concentration although it did not modify the kinetics or the last step (fusion of the secretory vesicles with the plasma membrane) of the exocytotic process.[32] Thus, catestatin exerts

its catecholamine release inhibitory effect by blocking the initial step (nAChR-mediated entry of Na^+ from the extracellular space into the cell) of nicotinic cholinergic signal transduction.

2.7 Specificity of catestatin as an inhibitor of nicotinic cholinergic catecholamine release among chromaffin granule co-transmitters

Because catestatin is co-released from catecholamine storage vesicles with ATP, catecholamines, NPY as well as its precursor prohormone CHGA upon exocytosis stimulated by efferent sympathetic outflow, we asked whether any other co-transmitters of catestatin exhibits catecholamine release inhibitory action.[34] We observed that, among the vesicle co-transmitters tested, only catestatin and neuropeptide Y (NPY) inhibited catecholamine secretion induced by nicotine, that catestatin is ~10-fold more potent than NPY, and that unlike catestatin NPY is not a specific antagonist of nicotinic cholinergic stimulated exocytosis because NPY also significantly blocked catecholamine secretion stimulated by membrane depolarization. Thus, catestatin emerges as a specific and potent inhibitor of nicotine-evoked catecholamine release among its chromaffin granule co-transmitters in the exocytotic cocktail.[34]

These *in vitro* studies in adrenal chromaffin cells and hippocampal neurons provided compelling evidence to suggest that catestatin acts as an antagonist at the nAChR to inhibit further release of catecholamines and its precursor protein CHGA by an autocrine negative feedback mechanism, which may ultimately modulate blood pressure and cardiac functions.

3. Catecholamine release inhibitory effect of catestatin: *ex vivo* and *in vivo* studies

3.1 In superfused rat adrenal glands

Catestatin efficiently blocked exocytotic release of catecholamines triggered *in situ* by nicotine from superfused rat adrenal glands.[29] In parallel experiments, catestatin also inhibited catecholamine secretion evoked by acetylcholine or by stimulation of splanchnic nerve.[29] Thus, consistent with *in vitro* studies in PC12 cells,[28,33,34] bovine chromaffin cells,[28] mouse chromaffin cells,[32] and hippocampal neurons,[24] catestatin demonstrated its catecholamine release inhibitory activity under *ex vivo* experimental conditions.

3.2 In mice

To test whether the *in vitro* and *ex vivo* catecholamine release inhibitory effect of catestatin also occurs *in vivo* in mice, we injected nicotine with or without pretreatment with catestatin. Acute nicotine treatment (~30 min) caused ~2.5-fold increase in the release of norepinephrine and epinephrine into the circulation, and catestatin blocked the response to nicotine.[37]

These findings suggest that catestatin may have a physiological role as an autocrine negative feedback inhibitor of catecholamine release. Therefore, catestatin may act as a physiological break for indiscriminate release of catecholamines into the circulation and exert anti-hypertensive property. Indeed, lower plasma level of catestatin has been associated with development of hypertension (see Section 7).[23]

4. Catestatin stimulates release of the vasodilator histamine from mast cells

Catestatin displayed potent vasodilatory effect in rat and this effect was mediated by augmented release of histamine[38] (see Section 5). Kruger *et al*.[39] hypothesized that the likely mechanism by which catesatin might trigger histamine release would be via stimulation of mast cells. They used the core active domain of bovine catestatin, $CHGA^{33}_{344-358}$ for this study. This peptide evoked ~60% release of total cell histamine at 5 μM concentration from peritoneal and pleural mast cells.[39] Moreover, the histamine releasing effect was dose-dependent (0.01–5 μM) with EC_{50} (peptide concentration eliciting half-maximal release of histamine) values of 0.6 and 0.9 μM for peritoneal and pleural mast cells, respectively.[39] Interestingly, catestatin was found to be more potent than the wasp venom peptide mastoparan in evoking histamine release from these mast cells.[39] In addition, catestatin displayed better efficacy than the other histamine-releasing peptides such as Substance P and Neurotensin in peritoneal and pleural mast cells.[39]

4.1 Physiological relevance

The most abundant sources of CHGA and catestatin, outside sympathoadrenal system may be the enterochromaffin-like and enterochromaffin cells of the gastrointestinal tract and polymorphonuclear neutrophils.[17,39] No data is available on what might be the free catestatin concentrations in the vicinity of mast cells under physiological conditions. However, the EC_{50} values of 0.6–0.9 μM for catestatin for stimulating histamine release from the peritoneal and pleural mast cells may be attainable in the cellular environment under such conditions that involve massive activation of the processing and release of this peptide from enterochormaffin cells and polymorphonuclear neutrophils accumulated during inflammation.[39] It is also noteworthy that mast cells have been implicated in the pathogenesis of a variety of cardiovascular diseases including arrhythmias, sudden cardiac death, myocardial dysfunction, and congestive heart failure,[40,41] although an effect of catestatin on cardiac mast cells is yet to be investigated.

4.2 Mechanism of action

The possible mechanism of action involves inhibitory G proteins (GTP-binding regulatory proteins) because catestatin-evoked histamine release was suppressed by the G_i inactivator pertussis toxin.[39] This mechanism is analogous to that already established for the cationic peptide mastoparan.[42] Moreover, in the peritoneal mast cell population, the catestatin response showed non-saturated kinetics, which is expected for a receptor-independent mechanism proposed for a wide range of cationic peptides.[17,39]

Thus, distinctly apart from being a potent non-competitive inhibitor of catecholamine release from sympathoadrenal system, catestatin may be one of the endogenous activators of histamine release from peritoneal and pleural mast cells that are devoid of acetylcholine receptors.

5. Cardiovascular effects of catestatin in animal models

5.1 Potent vasodilator and sympathoadrenal actions in rats

In view of the catecholamine release inhibitory effect of catestatin in chromaffin cells,[28] Kennedy *et al*. carried out experiments in rats to test cardiovascular effect of the peptide *in vivo*.[38] Intravenous injection of catestatin to attain an extracellular fluid concentration of ~6 μM diminished blood pressure responses to activation of sympathetic outflow by a series of 20 Hz electrical stimulations on pithed rats both during and after stimulations. Prior α and β adrenergic blockade [by phenoxybenzamine and propranolol, respectively] greatly reduced the size of acute pressor responses during electrical stimulation, but the prolonged post-stimulation pressor response was still present.[38] Interestingly, catestatin significantly (~40%) reduced the prolonged pressor response even in the adrenergically blocked rats.[38] As a control peptide, bovine $CHGA_{141-160}$ was used in these experiments and it was without an effect demonstrating specificity of catestatin.

A previous study documented that the acute phase of the blood pressure response to electrical stimulation results from catecholamine release while the prolonged phase of the response results from the action of NPY in this rat model.[43] Consistently, catestatin markedly blunted the pressor response to exogenous NPY and NPY receptor Y1 agonist in this study of catestatin effects *in vivo*.[38] But the persistent vasodepression after adrenergic blockade does not corroborate with catestatin's catecholamine release inhibitory effect. However, in this animal model, catestatin increased endogenous circulating histamine level by 21-fold. Intriguingly, the vasodepressor activitiy was mimicked by exogenous histamine and largely blocked by histamine H1 receptor antagonist hydroxyzine. Thus, the exogenous effect of catestatin in rats under those experimental conditions seems to be an indirect vasodilation mediated, at least in part, by histamine release involving H1 receptor.[38]

5.2 Alteration of blood pressure and cardiac physiology in mice

Recently, we generated a knockout mouse model by systemic deletion of *chga* gene.[15] These knockout mice ($chga^{-/-}$) displayed elevated blood pressures (~44 mmHg higher SBP and ~26 mmHg higher DBP) when compared with wild-type ($chga^{+/+}$) mice. To test whether infusion of exogenous catestatin results in a vasodepression, we injected human catestatin (to achieve a concentration of ~4 μM in the extracellular space) to $chga^{-/-}$ as well as $chga^{+/+}$ mice. Catestatin replacement in $chga^{-/-}$ mice resulted in a substantial reduction of elevated SBP toward the wild-type level. The SBP of $chga^{+/+}$ mice was also found to decrease after catestatin injection. Interestingly, the rate of pressure depression was faster for $chga^{-/-}$ mice when compared with $chga^{+/+}$ mice reflecting excellent hypotensive nature of this peptide.[15] This observation is in corroboration with a human study showing that hypertensive individuals contain lower plasma levels of catestatin when compared with normotensive controls.[23]

The blood pressure lowering action of catestatin may have resulted from its nicotinic cholinergic antagonistic activity as observed *in vivo* in mice[37] to act as a physiological break on transmitter release from the sympathochromaffin system. Indeed, the plasma catecholamine (both norepinephrine and epinephrine) levels in $chga^{-/-}$ mice (that did not have any catestatin because of the absence of the parent molecule) were approximately two-fold higher than those in $chga^{+/+}$ mice[15] consistent with this suggestion.

As described in Section 5.1, catestatin displayed vasodepression activity by stimulating histamine release in an experimental rat model.[38] It is not known whether similar phenomenon occurs in mice also because histamine levels in mice after catestatin injection was not measured. Therefore, it remains to be determined whether the hypotensive effect of catestatin in mice, at least in part, is secondary to histamine release from mast cells.

Of note, the elevated blood pressure in $chga^{-/-}$ mice was also rescued by humanization of mice at the chromogranin A locus (by generation of $chga^{-/-}CHGA^{+/+}$ mice that contain two copies of human *CHGA* gene) implying a hypotensive effect of CHGA-derived catestatin.[15] However, because the prohormone chromogranin A also gives rise to the vasoactive peptide vasostatin[26] by proteolytic cleavage, the elevated blood pressure in $chga^{-/-}$ mice and rescue of hypertension by expression of human CHGA may have been resulted/contributed by vasostatin also. Knockout mouse models specifically deleting the catestatin and vasostatin regions may provide better insight into the mechanism of development of hypertension in these mice.

In view of high blood pressure in $chga^{-/-}$ mice, we studied the left ventricular (LV) morphology in these mice at ~5 months of age.[15] Significant increases (in comparison with $chga^{+/+}$ littermates) in LV wall thickness (both septal and free wall) and a pronounced increment in estimated LV mass were detected. We also found significant increases in LV internal diameter (cavity size) at both end systole and end diastole. Although we did not investigate whether such alteration of cardiac physiology was solely due to catestatin deficiency, but it is conceivable that substantially increased afterload because of hypertension in these chromogranin A-null (and hence catestatin-deficient) mice would lead to the LV hypertrophy and progression to LV cavity dilation.

6. Naturally occurring human variants of catestatin differ in potency

During the course of investigation of polymorphisms in the *CHGA* locus in 180 people of urban Southern California, we discovered three non-synonymous single nucleotide polymorphisms in the catestatin region: Gly364Ser, Pro370Leu, and Arg374Gln.[33,44] The Gly364Ser polymorphism alters a site that is otherwise absolutely conserved among mammals; the Pro370Leu polymorphism was a reversion of the wild-type human amino acid Pro370 to Leu370 seen in all non-primate mammals; and the Arg374Gln polymorphism disrupted the usual dibasic processing site Arg374Arg flanking the C-terminus of this peptide.[33] We synthesized these naturally occurring catestatin variants and tested for their relative abilities to inhibit nicotinic cholinergic stimulated catecholamine secretion in PC12 cells. The Gly364Ser and Arg374Gln variants were ~4.5- and ~27-fold less potent, respectively, while the Pro370Leu variant was ~2.2-fold more potent in comparison with the wild-type catestatin[33,44] (*Figure 3*). Future studies

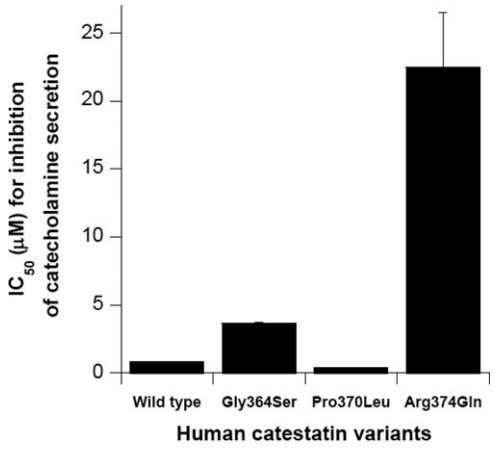

Figure 3 Comparative potency of human catestatin variants. Catecholamine secretion evoked by nicotine from PC12 cells was monitored in the absence and presence of ascending doses (0.1–10 μM) of human catestatin variants. IC_{50} value for inhibition of catecholamine secretion was calculated and plotted against each peptide. The variants displayed altered potency to inhibit nicotine-evoked catecholamine secretion demonstrating functional significance of the amino acid substitutions. Data for generating this plot was taken from Mahata *et al.*[33]

may unfold *in vivo* implications of such substantial (~60-fold) differences in potency among these naturally occurring variants of catestatin.

The mechanistic basis of difference in the potency among the catestatin variants is not clear. Nevertheless, molecular modelling suggested that Gly364Ser substitution caused distortion of the peptide backbone in two locations, by 0.36 Å in the middle loop near the site of substitution and by 1.48 Å in the C-terminal strand, which is adjacent in the 3D structure; the Pro370Leu substitution caused a 0.59 Å shift in the middle loop but no shift in the C-terminal strand near the site of substitution.[44] Such structural alterations may have contributed to the variation in potency. Another possible explanation stem from studying the Hill plots monitoring the fractional effect of catestatin to inhibit nicotine-triggered catecholamine release.[33] Decline in Hill slope was found for less potent variants suggesting that such catestatins might actually exhibit negative co-operativity at ascending peptide doses. This may happen because catestatins exhibit amphiphilic β sheet character in solution, with alternating cationic and hydrophobic amino acid residue side chains, wherein the hydrophobic domains may contribute to formation of a hairpin (β-strand/loop/β-strand) structure by intramolecular (strand-to-strand) hydrophobic collapse,[18,45] and intermolecular hydrophobic interactions might result in self-association leading to diminution in the effective local concentration of the active monomeric peptide.[33] Further studies are required to confirm these suggestions.

7. Catestatin effects on blood pressure and cardiac functions in humans

7.1 Plasma catestatin levels inversely correlate with hypertension

In view of the antagonistic activity of catestatin at the nAChR, the physiological trigger to efferent autonomic outflow, a study was undertaken to explore whether catestatin level in circulation is altered in hypertensive individuals.[23] This study involving 61 hypertensive and 216 normotensive people of San Diego, CA, revealed that plasma catestatin level was diminished not only in established hypertensives but also in their still-normotensive offspring.[23] In fact, family history was found to have a major influence: despite having similar blood pressures, normotensive subjects with positive family history displayed substantially lower catestatin level than their counterparts having negative family history.[23] Thus, the decline in plasma catestatin seems to be a very early event (even pre-hypertensive) in the course of development of hypertension; such early decline in the plasma level suggests a pathophysiological role of catestatin in the development of hypertension rather than a late response to the disease state.

Of note, African-American hypertensive patients with End Stage Renal Disease also have diminished levels of plasma catestatin.[46] Do such patients display low plasma catestatin as well much before the target organ complication phase? Future studies may answer this question.

In addition to diminished catestatin in circulation, individuals with positive family history of hypertension had greater (approximately two-fold elevation when compared with those with negative family history) urinary epinephrine excretion suggesting an *in vivo* inhibitory effect of catestatin on catecholamine release.[23] This finding is complementary to the previous report that the resting arterial plasma norepinephrine concentration was ~1.7-fold higher in normotensive offspring of hypertensive patients than in normotensive subjects with no family history of essential hypertension.[3]

Consistent with the apparent tonic sympathoinhibitory effect of catestatin, subjects with lower plasma catestatin had magnified diastolic and mean blood pressure elevations to cold stress (immersion of the left hand in ice water for 60 s after a 10 min rest) when compared with subjects with higher plasma catestatin.[23] Such enhanced pressor responses to a sympathoadrenal stressor in subjects with catestatin deficiency suggest an adrenergic mechanism whereby diminished circulatory catestatin may lead to development of hypertension in future.[23] Thus, lower plasma catestatin emerges as an 'intermediate phenotype' for ultimate hypertension disease states.

7.2 Exogenous catestatin increases forearm blood flow

Arterial infusion of human catestatin (at 100 nmole/min into brachial artery; target local concentration of 1 μM) in the forearm of 14 healthy human subjects caused a modest but significant enhancement of blood flow.[47] This vasodilation is consistent with the catecholamine secretion-inhibitory[28] and histamine release-stimulating[39] activities of catestatin, although which of these mechanisms is responsible for this effect remains unknown. However, this catestatin effect seems to be specific because another CHGA-derived peptide pancreastatin[25] (*Figure 1*) did not alter the forearm blood flow under identical experimental conditions.[47]

7.3 Human catestatin variant Gly364Ser alters cardiac activity and risk for hypertension

Among the naturally occurring human variants of catestatin (see Section 6), the Gly364Ser variant causes profound changes in cardiac activity as observed in a Southern

California population.[24] The Gly/Ser heterozygotes displayed increased baroreceptor slope during upward and downward deflections (by ~47 and ~44%, respectively), increased cardiac parasympathetic index (by ~2.4-fold), and decreased cardiac sympathetic index (by ~26%) when compared with the Gly/Gly homozygotes.[24]

The increased baroreceptor sensitivity in these normotensive individuals with 364Ser allele may render protection against future development of hypertension in view of the following observations: (i) baroreceptor activity is diminished not only in hypertensive but also in still-normotensive offspring of hypertensive patients,[48] (ii) chronic activation of the baroreflex produces sustained reductions in blood pressure and sympathetic activity in animals.[49] Consistently, after environmental (cold) stress, the pressor response was significantly less in the Gly/Ser heterozygotes when compared with the Gly/Gly homozygotes.[24]

The Gly364Ser genotype also exerted significant effects on resting blood pressure: Gly/Gly homozygotes displayed ~5–6 mmHg higher DBP than Gly/Ser heterozygotes in two independent study populations; the SBP was also ~13 mmHg higher in individuals with Gly/Gly genotype when compared with those with Gly/Ser genotype in one population.[24] Interestingly, this lower blood pressure effect for 364Ser genotype was confined to men although the mechanism of such sex-dependent genotype effect remains unknown. Thus, the 364Ser genotype seems to reduce risk of developing hypertension, especially in men.[24] Of note, the renal norepinephrine and epinephrine excretions were significantly lower in the 364Ser carriers. The plasma norepinephrine was also lower in these individuals when compared with the Gly/Gly homozygotes.[24] Such diminished catecholamine secretion is consistent with the lower blood pressure in Gly364Ser heterozygotes.

What might be the mechanism for the changes in parasympathetic and sympathetic activities as well as an effect on blood pressure in the human carriers of 364Ser catestatin? The central nicotinic-cholinergic synapses[50] in the nucleus of the tractus solitarius (NTS) in the cardiovascular/baroreceptor control region of the brain stem act to enhance baroreceptor activity, increasing parasympathetic while decreasing sympathetic activity.[24] It has been proposed that catestatin may act at these NTS synapses since Gly364Ser genotype is associated with such reciprocal changes in autonomic efferent activity and CHGA, the precursor of catestatin, found in the brain stem.[24] Future studies may establish the mechanism for such alteration of autonomic activity and blood pressure in individuals carrying 364Ser variant of catestatin.

8. Conclusions and perspectives

Increased sympathoadrenal tone including plasma catecholamine elevations plays an early pathogenic role in augmentation of blood pressure. Recent studies have documented that the CHGA-derived peptide catestatin acts as a novel and potent autocrine modulator of catecholamine secretion in chromaffin cells and adrenergic neurons in isolated cells as well as in intact organisms. Therefore, this endogenous catecholamine-release inhibitory peptide is a logical regulator of blood pressure and cardiac functions. Indeed, the plasma level of catestatin is diminished early in the course of development of hypertension, even in the normotensive offspring of hypertensive patients suggesting involvement of this peptide in pathogenesis of the disease. Lower circulating catestatin level also predicts augmented adrenergic pressor responses to environmental stress indicating that diminished catestatin level might be a risk factor for development of hypertension. Consistent with these findings in human studies, exogenous catestatin reduces blood pressure in experimental animals. Three naturally occurring human variants of catestatin (Gly364Ser, Pro370Leu, and Agr374Gln) displaying differential potencies for inhibition of catecholamine secretion were identified in a Southern California population. Among these variants, the Gly364Ser catestatin peptide caused increased baroreceptor sensitivity, increased cardiac parasympathetic activity, decreased sympathetic index, and seemed to alter the risk for hypertension in humans. It would be of interest to investigate whether the 364Ser allele imparts such cardio-protective effect also in other human populations for possible clinical applications. The mechanisms emerged thus far for these cardiovascular effects of catestatin include autocrine inhibition of catecholamine release from the sympathoadrenal system, paracrine stimulation of the potent vasodilator histamine release from mast cells, and modulation of both sympathetic and parasympathetic activities by acting at the baroreceptor centre of the NTS (*Figure 4*). Further studies (such as whether catestatin plays a role in salt-sensitive hypertension, which involves hyper-sympathetic activity regulated by endothelin-1[51] and angiotensin-II[52,53] as well as increased sensitivity to norepinephrine[54]) looking at the functions and the underlying mechanisms of action of this physiological antihypertensive peptide may yield important insights into the pathogenesis of cardiovascular disease states.

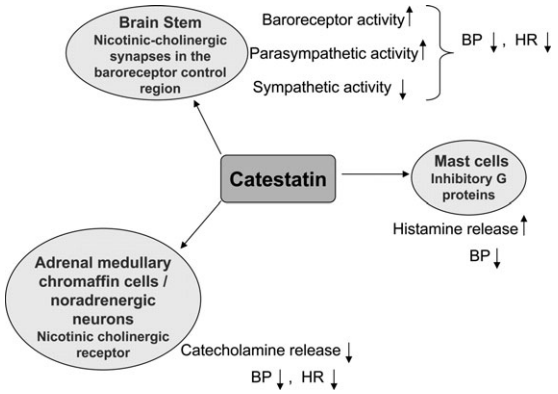

Figure 4 Mechanisms of action of catestatin. A schematic of three possible mechanisms by which catestatin may exert its cardiovascular effects is shown. The catestatin peptides act on (i) nicotinic acetylcholine receptors on adrenal medullary chromaffin cells or adrenergic neurons to inhibit catecholamine secretion, (ii) peritoneal and pleural mast cells involving inhibitory G-proteins (GTP-binding regulatory proteins) to stimulate release of the vasodilator histamine, (iii) central nicotinic-cholinergic synapses in the nucleus of the tractus solitarius in the baroreceptor control region of the brain stem to enhance baroreceptor activity, increase parasympathetic activity, and decrease sympathetic activity. These activities may give rise to the blood pressure- and heart rate-lowering functions of this endogenous peptide.

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