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Development of a pharmacophore model for the catecholamine release-inhibitory peptide catestatin: Virtual screening and functional testing identify novel small molecule therapeutics of hypertension

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ABSTRACT

The endogenous catecholamine release-inhibitory peptide catestatin (CST) regulates events leading to hypertension and cardiovascular disease. Earlier we studied the structure of CST by NMR, molecular modeling, and amino acid scanning mutagenesis. That structure has now been exploited for elucidation of interface pharmacophores that mediate binding of CST to its target, with consequent secretory inhibition. Designed pharmacophore models allowed screening of 3D structural domains. Selected compounds were tested on both cultured catecholaminergic cells and an in vivo model of hypertension; in each case, the candidates showed substantial mimicry of native CST actions, with preserved or enhanced potency and specificity. The approach and compounds have thus enabled rational design of novel drug candidates for treatment of hypertension or autonomic dysfunction.

1. Introduction

Hypertension is the most common and lethal of cardiovascular risk factors,[1] yet despite pharmacological advances, it remains only partially controlled by antihypertensive medications.[2] Here we targeted the novel catecholamine storage/release hormone catestatin for therapeutic potential, by analysis of its pharmacophore features, yielding a family of small organic compounds with preserved potency and pathway specificity.

Chromogranin A (CHGA, OMIM 118910), is the 48 kDa protein found in catecholamine secretory vesicles of chromaffin cells and postganglionic sympathetic axons.[3–7] CHGA contains characteristic sites for proteolytic cleavage[4] by which it is transformed to biologically active peptides: pancreastatin ($hCHGA_{250–301}$),[8–10] prochromacin ($bCHGA_{79–431}$),[11] vasostatin ($hCHGA_{1–76}$),[12] and catestatin (CST: bovine $CHGA_{344–364}$: RSMRLSFRARGYGFRGPGLQL; human $CHGA_{352–372}$: SSMKLSFRARGYGFRGPGPQL),[13,14] a well characterized inhibitor of catecholamine release[14] working as antagonist at neuronal nicotinic acetylcholine receptors.[15] In human patients with hereditary hypertension, or their offspring, the concentration of CST in the plasma is diminished, suggesting that its deficiency can play a pathogenic role in development of hypertension.[16–18] Targeted ablation of the CHGA locus in the mouse results in unbridled hypertension[19] that can be 'rescued' by administration of CHGA's catecholamine release-inhibitory catestatin fragment.[19] The catestatin fragment of CHGA exerts both antihypertensive[19–21]

Abbreviations: 3D, three-dimensional; AchR, acetylcholine receptor; ASA, water-accessible surface area; BP, blood pressure; CHGA, chromogranin A; CST, catestatin; DBP, diastolic blood pressure; DSI, Data Sciences International (St. Paul, MN); HR, heart rate; NMR, nuclear magnetic resonance; SBP, systolic blood pressure; SEM, standard error of the mean.

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and vasodilatory[22,23] actions in vivo, in both rodents and humans. These observations render catestatin an attractive template for pharmacophore-based drug design.

Earlier we solved the structure of catestatin (CST) with NMR spectra, in which CST assumed the 3D conformation of a twisted loop.[24] Using Ala-scanning mutations, we elucidated the residues that are most important in CST inhibition of catcholamine release at the nicotinic acetylchoine receptor (AchR).[25] The impact of mutations P370L and G364S of CST to its interaction with α3β4 nAChR have been modeled by Sahu et al.,[26] showing that mutation P370L increases interaction of CST with nAChR, while the mutation G364S decreases this interaction. Based on our NMR and Ala-scanning results,[24,25] we defined probable intermolecular contacts between CST and the AchR and the most important CST residues for this interface. The 3D map of important residues and side chains was then used for design of a pharmacophore hypothesis utilized by compounds that would mimic CST actions at the AchR. This analysis led us to a six-feature pharmacophore hypothesis. This hypothesis was used for 3D search of the Open NCI and commercial 3D databases. The majority of the selected compound fit to five-of-six features of this hypothesis. Seven of the selected compounds have shown significant CST mimicking properties.

We tested compounds' potency, efficacy, and mechanistic specificity for inhibition of cellular events triggered by nicotinic cholinergic stimulation, as well as antihypertensive activity in vivo. Our results suggest that synthetic catestatin small molecule analogs can be designed with potency and specificity for nicotinic cholinergic-stimulated catecholamine release, ultimately yielding activity as antihypertensive agents in vivo.

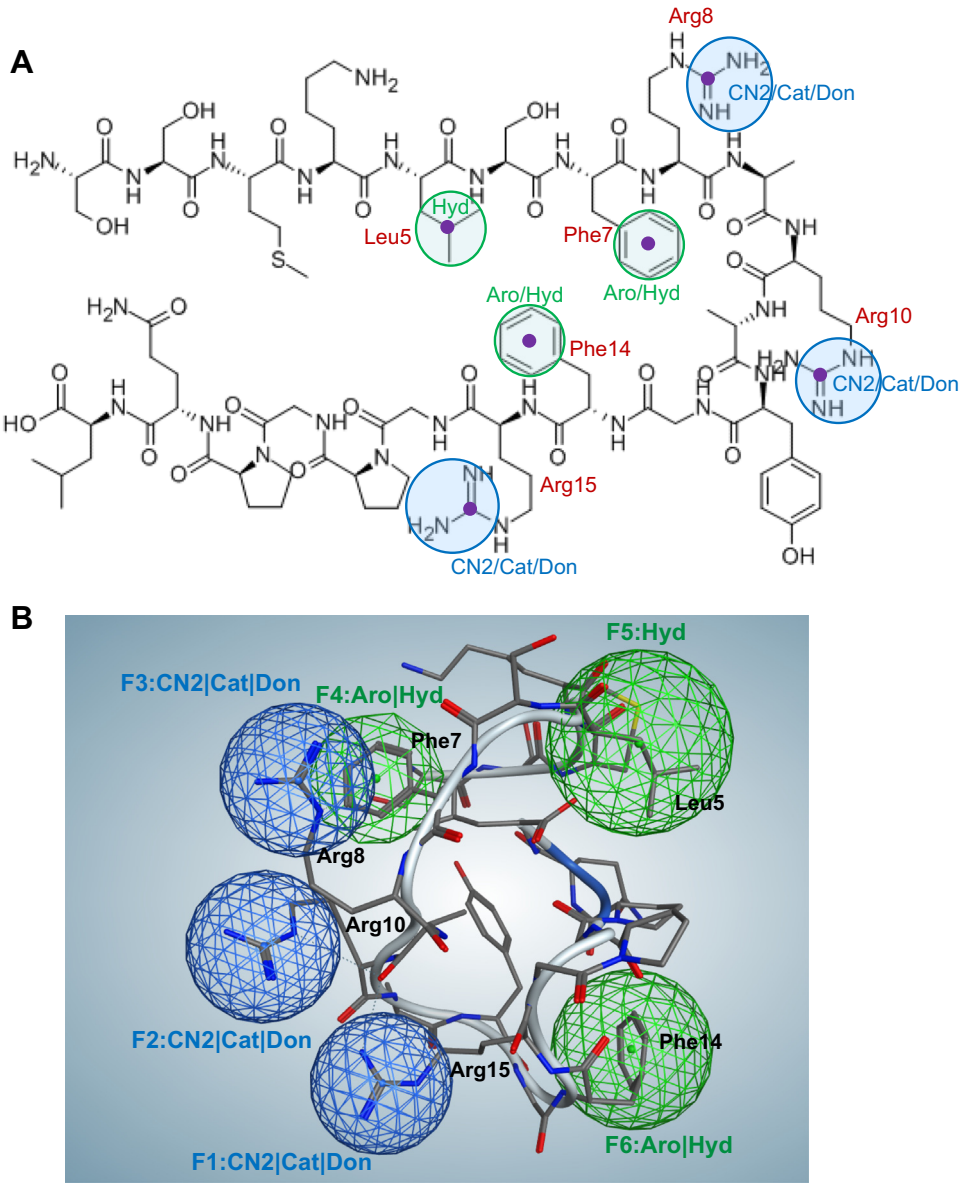

Figure 1. Catestatin structure and pharmacophore hypothesis. (A) Primary structure (amino acid sequence) of catestatin with pharmacophore annotation points (purple dots) and pharmacophore features (green and dark-blue circles). Annotation points correspond to hydrophobic Leu5, Phe7, and Phe14; and positively charged Arg8, Arg10, and Arg15. Green circles represent hydrophobe and aromatic/hydrophobic features, while dark-blue circles represent NCN^{+} groups/cations/H-bond donors. (B) Catestatin pharmacophore hypothesis corresponding to annotation shown in A; the color codes are the same. Ribbon diagram and three-dimensional residue structures belong to superimposed catestatin.

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