Characterization of Novel Unimolecular Amylin-Adrenomedullin Dual Agonists

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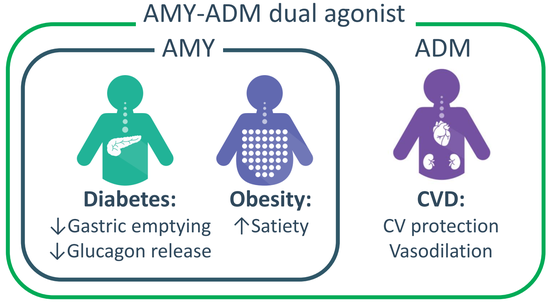
Introduction

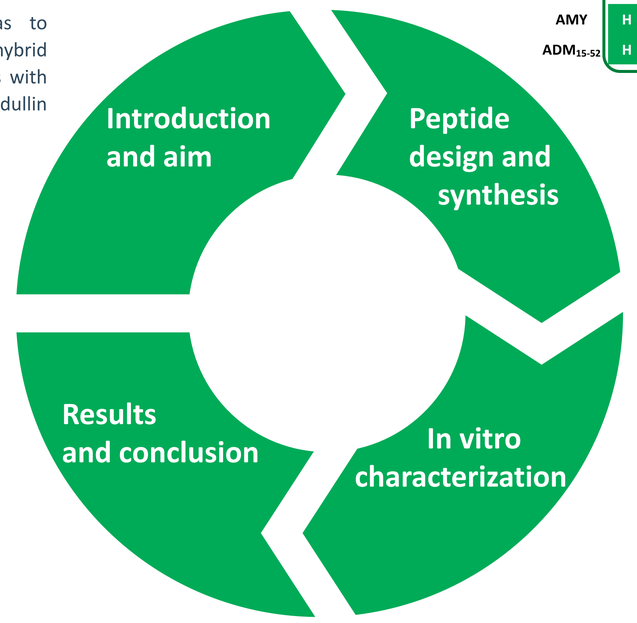
Amylin and Adrenomedullin both belong to the calcitonin peptide family. While amylin plays an important role in maintaining glucose and energy homeostasis, adrenomedullin is primarily known for its beneficial vasoactive effects. We hypothesize that combining the glucoregulatory and anti-obesity properties of amylin with the cardioprotective effects of adrenomedullin could be an effective treatment strategy to address type 2 diabetes, obesity and cardiovascular diseases (CVD) in one molecule.



Aim

The aim of the present study was to synthesize and characterize novel hybrid amylin-adrenomedullin peptide agonists with dual activity at the amylin and adrenomedullin receptors.

Figure 1: The major therapeutic potential of amylin (AMY), and adrenomedullin (ADM). An AMY-ADM dual agonist would target all indications in one molecule.



Strategy for synthesis of amylin-adrenomedullin dual agonists

Amylin and Adrenomedullin have overlapping sequences and highly conserved structural features including a disulfide-bonded ring structure and an amidated C-terminal tyrosine. This allow for rational design of chimeric dual amylin-adrenomedullin agonists (Figure 2). Peptides were synthesized using solid-phase peptide synthesis. The pharmacokinetic profile was improved by N-terminal lipidation.

Figure 2: Sequence alignment of amylin (AMY) and Adrenomedullin (ADM)

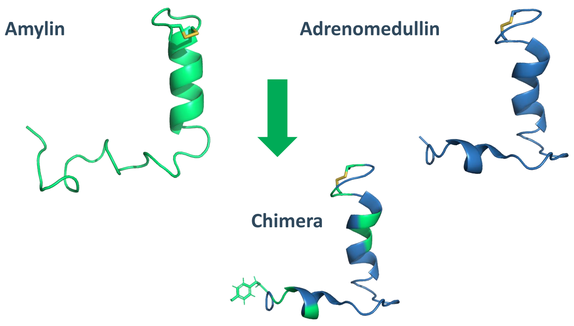
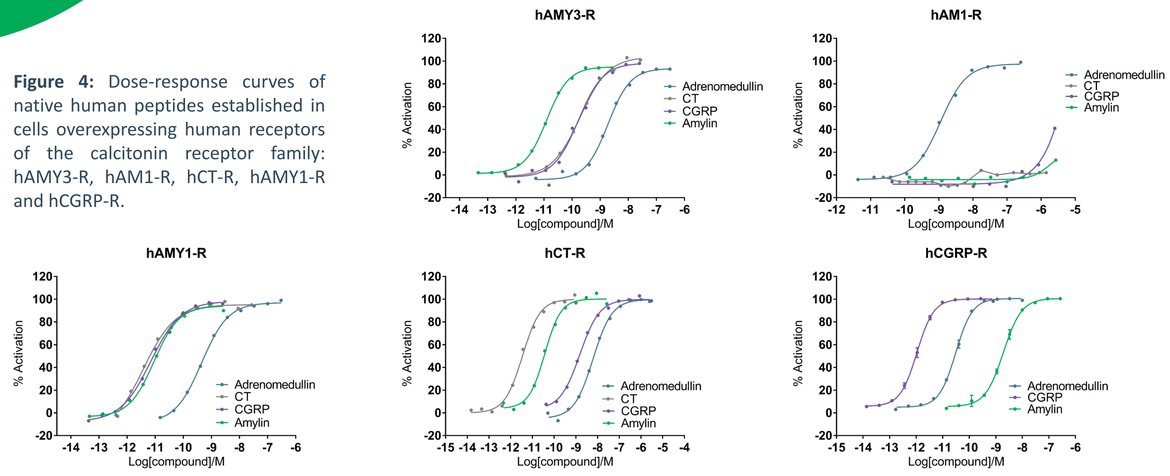


Figure 3: NMR structure of amylin (Green) and adrenomedullin (Blue). Chimeric peptides are based on the adrenomedullin (15-52) backbone with different combinations of amylin residues.

In vitro characterization

Functional activities of the amylin-adrenomedullin dual agonists were measured using a cAMP accumulation assay in cells stably overexpressing human amylin receptor subtype 3 (hAMY3-R) or human adrenomedullin receptor subtype 1 (hAM1-R). Counter screening was performed in cells overexpressing additional receptors of the calcitonin receptor family members: hCGRP-R, hCT-R and human hAMY1-R.

Figure 4: Dose-response curves of native human peptides established in cells overexpressing human receptors of the calcitonin receptor family: hAMY3-R, hAM1-R, hCT-R, hAMY1-R and hCGRP-R.



Amylin-adrenomedullin dual agonists dual agonists

All analogues were full agonists on both receptors. hAMY3R potencies were comparable to human native amylin, whereas hAM1-R potencies ranged from equipotent to 100-fold less potent compared to human native adrenomedullin.

Compound	AMY3-R EC_{50} (nM)	AM1-R EC_{50} (nM)
Amylin	0.01	>1000
$ADM_{(15-52)}$	0.929	1.7
Lipid. $ADM_{(15-52)}$	0.57	3.9
(1)	0.117	4.8
(2)	0.129	8.3
(3)	0.126	50
(4)	0.185	58
(5)	0.133	310
(6)	0.056	8.4

Table 1: In vitro potency of lipidated amylin-adrenomedullin dual agonists at hAMY3-R and hAM1-R.

Perspective

Future directions include addressing the in vivo efficacy and potency of the amylin-adrenomedullin dual agonists in rodent models of obesity, diabetes and cardiovascular diseases. We will use standard diet induced rodent model for addressing the anti-obesity effects. The CV protective effects will be assessed in the left anterior descending artery (LAD) ligation model.

Abbreviations: AMY: Amylin, ADM: Adrenomedullin, hAMY3-R: human amylin receptor subtype 3, hAM1-R: human adrenomedullin receptor subtype 1, hCGRP-R human calcitonin gene related peptide receptor, hCT-R: human Calcitonin receptor, CVD: cardiovascular diseases.

Conclusion

We here report for the first time the synthesis and characterization of novel chimeric amylin-adrenomedullin peptide agonists with dual activity at the hAMY3-R and hAM1-R for the potential treatment of obesity patients with high cardiovascular risk.