https://doi.org/10.17952/37EPS.2024.P2200

Pipeline for development of acylated peptide-based CGRP receptor antagonist with extended half-life for migraine treatment

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Background & Aim

Migraine is a debilitating headache disorder. The disease has neurovascular origin and migraine attacks can be elicited by vasodilative neuropeptides such as alpha calcitonin gene-related peptide (αCGRP). Antagonizing αCGRP actions in migraine patients has proven clinically efficient. Here, we present a pipeline for development of a peptide-based αCGRP receptor antagonist with increased half-life capable of antagonising the vasodilatory effect of αCGRP.

Methods

A series of αCGRP8-37 analogues carrying a C18-or C20-diacid lipidation were screened in vitro for CGRP receptor antagonism. An αCGRP8-37 analogue protracted with a C20 diacid at position 25 was subjected to an amino acid substitution screen to identify mutations that could further enhance antagonism. Average purity of peptides included in the lipidation scan, and the mutation scan ranged between 30-50%. Findings from lipidation scan and mutation scans were subsequently confirmed by characterising potency of selected antagonists with a purity of >95 %.

Conclusion

+ Antagonism of αCGRP8-37 is maintained with C20DA-γGlu protraction at N25K.
+ A ~4-fold gain of potency is observed by substituting alanine with serine at position 36 increase.
+ αCGRP8-37 analogues carrying a C20 diacid lipidation antagonized vasodilative actions of αCGRP

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1 Lipidation scan

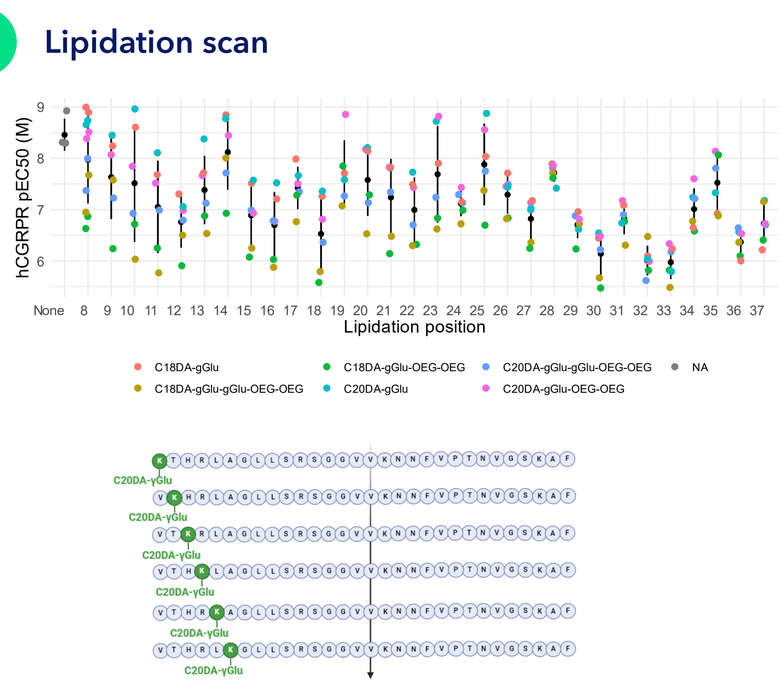

Figure 1. Greatest losses of potency were observed in the C-terminal region of αCGRP8-37. Potency might be maintained with N25K-C20DA-γGlu protraction.
Six lipidation moieties were applied to a lysine substitution at every amino acid residue of αCGRP8-37. A γGlu linker connects the peptide to C18DA or C20DA. The peptides were incubated with a fixed concentration of αCGRP corresponding to EC90 of αCGRP. Five-point concentration response curves were generated with antagonist concentrations of 30 nM, 100 nM, 300 nM, 1 µM and 3 µM. Antagonist potency was determined measuring cAMP applying HTRF Technology, CisBio. Black dots represent means. Error bars denote standard deviations.

2 Validation of lipidation

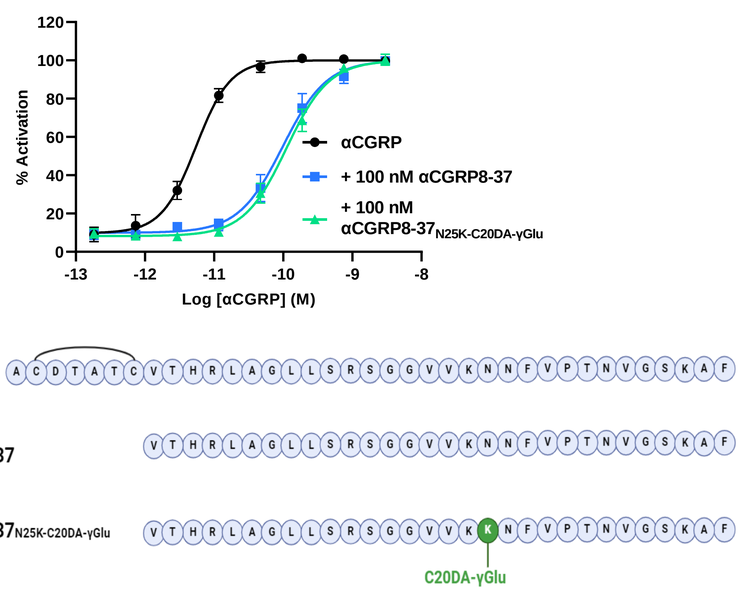

Figure 2. Antagonism of αCGRP8-37 is maintained with N25K-C20DA-γGlu protraction.
Inhibition of αCGRP-stimulated cAMP generation using a hCGRP-R CHO-K1 stable cell system. Concentration-response curves of αCGRP were generated in the absence or presence of 100 nM αCGRP8-37 or αCGRP8-37$_{N25K\text{-}C20DA\text{-}\gamma Glu}$.

3 Mutation scan

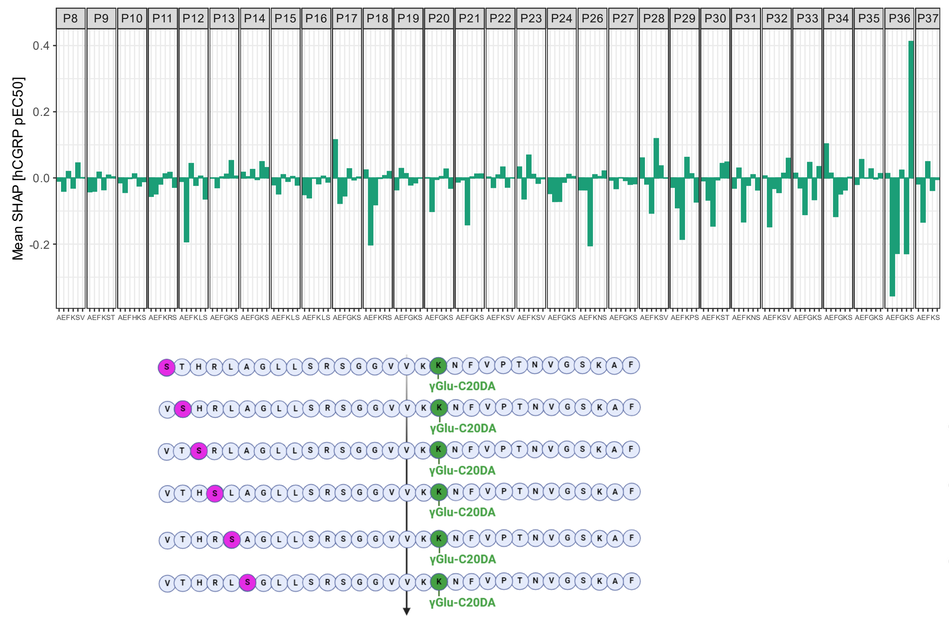

Figure 3. Mutations in the C-terminal region is generally associated with losses of potency. Serine at position 36 might induce a gain of potency.
Antagonist potency was evaluated with the cAMP HTRF technology (CisBio) applying five-point concentration response curves (30 nM to 3 µM). Each position was mutated to five different amino acids, either alone or in combination, resulting in a total data set consisting of 950 peptides. Lysine (K), glutamic acid (E), phenylalanine (F), serine (S), and alanine (A) were applied as substitutions. A random forest model was computed based on the relationship between the pEC50 values and the amino acid sequences. SHAP values were computed to determine the potency contribution of each mutation, where a positive SHAP value correspond to a positive contribution and a negative SHAP value corresponds to a negative contribution.

4 Validation of amino acid mutation

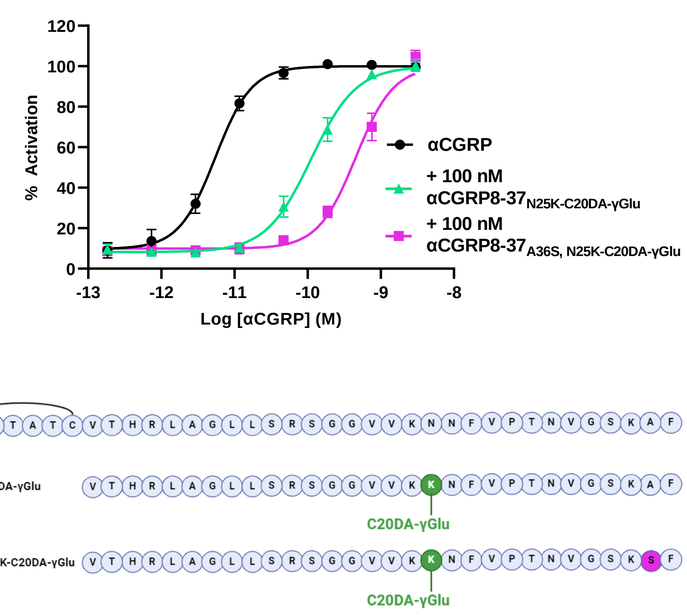

Figure 4. Serine at position 36 results in a 4-fold gain of potency to αCGRP8-37$_{N25K\text{-}C20DA\text{-}\gamma Glu}$.
Inhibition of αCGRP-stimulated cAMP generation using a hCGRP-R CHO-K1 stable cell system. Concentration-response curves of αCGRP were generated in the absence or presence of 100 nM αCGRP8-37$_{N25K\text{-}C20DA\text{-}\gamma Glu}$ or αCGRP8-37$_{A36S,\ N25K\text{-}C20DA\text{-}\gamma Glu}$.

5 Demonstration of ex vivo efficacy

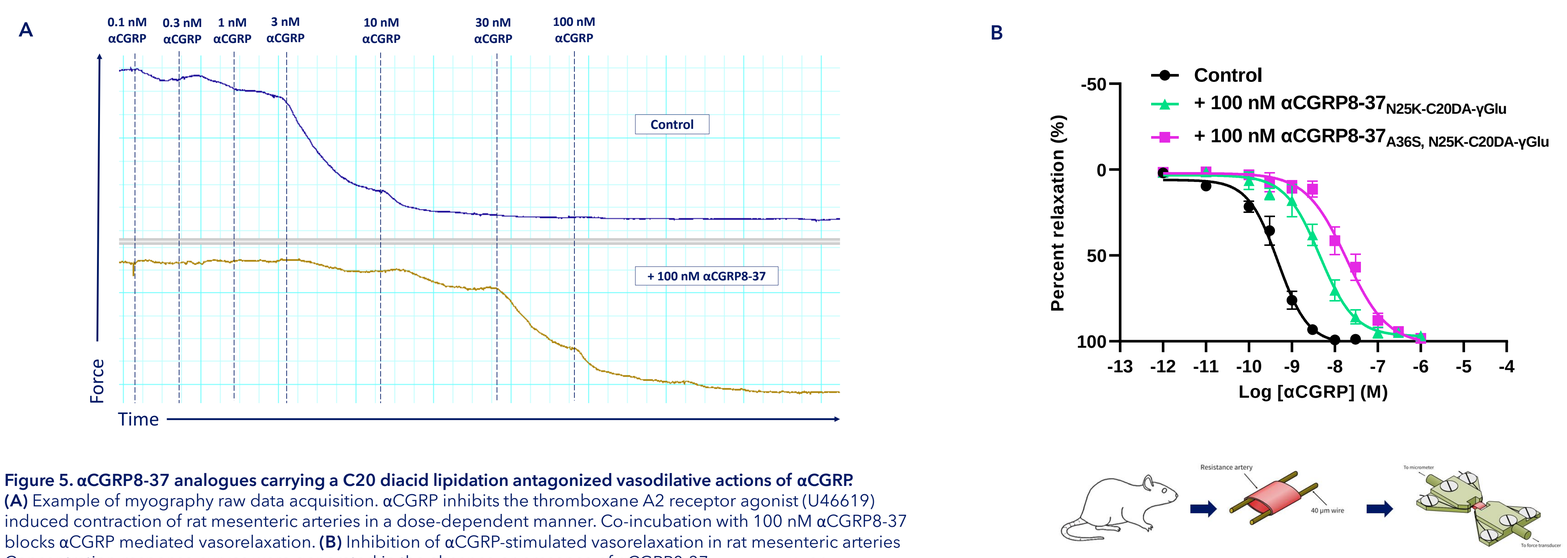

Figure 5. αCGRP8-37 analogues carrying a C20 diacid lipidation antagonized vasodilative actions of αCGRP.
(A) Example of myography raw data acquisition. αCGRP inhibits the thromboxane A2 receptor agonist (U46619) induced contraction of rat mesenteric arteries in a dose-dependent manner. Co-incubation with 100 nM αCGRP8-37 blocks αCGRP mediated vasorelaxation. **(B)** Inhibition of αCGRP-stimulated vasorelaxation in rat mesenteric arteries Concentration-response curves were generated in the absence or presence of αCGRP8-37$_{N25K\text{-}C20DA\text{-}\gamma Glu}$ or αCGRP8-37$_{A36S,\ N25K\text{-}C20DA\text{-}\gamma Glu}$.