Novel Analogs of the Mitochondrially Encoded Peptide CB5064 Improve Body Weight and Glucose Tolerance in DIO Mice, and Demonstrate Selective Agonism at the Apelin Receptor

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ABSTRACT

Metabolic dysregulation is integral to the development and pathogenesis of many age-related diseases, including type 2 diabetes and obesity. Several mitochondrially encoded peptides have previously been shown to be secreted from cells and to act as regulatory signals for metabolic homeostasis, including MOTS-c, SHLP2, and humanin. Novel modified analogs of a newly identified mitochondrially encoded peptide, MDP064 (CB5064), have been synthesized and evaluated for plasma stability, activity in a broad screen of G protein-coupled receptor (GPCR) target engagement, and for potential biological activity in diet-induced obese (DIO) mice. *In vitro* evaluation demonstrated that CB5064 analogs interact with and activate the apelin receptor (APJ/AGTRL1/APLNR). The observed EC50 values of several novel CB5064 analogs at the apelin receptor were in the low μM range, in some cases achieving maximum responses similar to the natural substrate, Apelin-13. In DIO mice, once daily administration of the same series of CB5064 analogs demonstrated a range of effects on metabolic regulation, including significant reduction in body weight, selective fat mass loss, and improved glucose tolerance. The apelin receptor is widely expressed in tissues and appears to play a critical role in energy metabolism, cardiovascular function, fluid homeostasis, angiogenesis, and in diabetic complications. These mitochondria-based peptide analogs therefore represent an entirely new class of molecules with agonist activity at the apelin receptor and potential for use as a source of novel therapeutics for type 2 diabetes, cardiovascular disease, and other age-related disorders. Additional evaluation of the therapeutic potential of these peptides is ongoing.

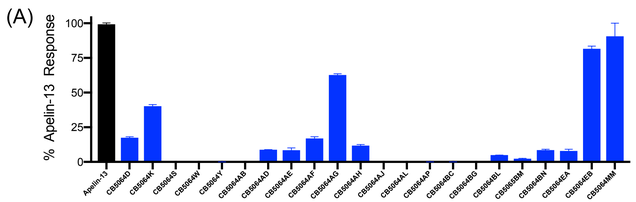
METHODS

β-Arrestin Recruitment Assay: β-Arrestin recruitment assays were performed by Eurofins-DiscoverX (Fremont, CA) using CHO-K1 AGTRL1 β-Arrestin cell line and PathHunter detection kit. Cells were seeded onto 384-well plates in standard medium. After overnight culture, the medium was replaced with buffer containing Apelin-13 (positive control) or CB5064 analogs. Following 90 min incubation at 37°C, β-Arrestin recruitment in response to various treatments was quantified.

cAMP Assay: cAMP accumulation in CHO-K1 AGTRL1 Gi cells (Eurofins-DiscoverX) was measured using HitHunter cAMP assay kit according to manufacturer's protocol (Eurofins-DiscoverX). Briefly, CHO-K1 AGTRL1 Gi cells were seeded onto 384-well plates in standard culture medium. After overnight culture, the medium was replaced with buffer containing forskolin (to increase cAMP expression) and either Pyr-Apelin-13 (inhibits cAMP accumulation) or CB5064 analogs. Following 30 min incubation at 37°C, cAMP levels in response to various treatments were quantified.

DIO Mouse Studies: CB5064 analogs (5 mg/kg) were administered by intraperitoneal (IP) dosing once daily for 10 days to 28 week old male DIO mice maintained on a high-fat diet (60% kcal from fat). Assessments: body weight, blood glucose (BG), fat and lean mass by qNMR, and food intake compared to vehicle control. For acute glucose tolerance test, blood glucose levels were monitored in overnight-fasted DIO mice following IP glucose (1.5 g/kg).

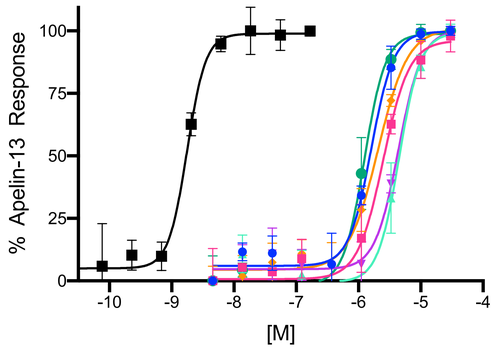
RESULTS



(B)

Peptide	% Apelin-13 Response	
	β-Arrestin Recruitment	Inhibition of cAMP Production
Apelin-13	100	100
CB5064D	17	64
CB5064K	40	105
CB5064AB	0	4
CB5064AD	9	27
CB5064AE	8	61
CB5064AF	17	8
CB5064AG	63	101
CB5064AH	12	53
CB5064AP	0	6
CB5064BL	5	1
CB5064BM	2	27
CB5064BN	9	21
CB5064EA	8	33
CB5064EB	82	44
CB5064MM	91	109

Figure 1: Response of human APJ overexpressing cell models to Apelin-13 (500 nM) or CB5064 peptide analogs (10 μM). (A) β-Arrestin recruitment assay. Data are mean (SD) percent of Apelin-13 response. (B) Comparison of β-Arrestin recruitment and cAMP accumulation assay formats. Data are percent of Apelin-13 response. Data are mean of n=2-3 for all data points.



Peptide	IC50 [M]
Apelin-13	1.763e-009
CB5064K	4.492e-006
CB5064AG	1.602e-006
CB5064AH	2.499e-006
CB5064EA	4.382e-006
CB5064EB	2.069e-006
CB5064MM	1.243e-006

Figure 2: Inhibition of cAMP accumulation in CHO-K1 AGTRL1 Gi cells following 30 min incubation with forskolin (10 μM) and Apelin-13 (0.025-167 nM) or CB5064 analogs (0.005-30 μM); data plotted as percent Apelin-13 response. Data are mean (SD) n=2-3 for all data points.

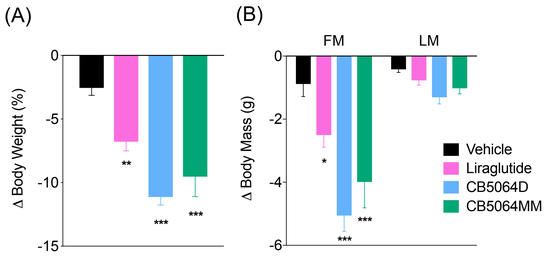


Figure 3: Effect of CB5064 analogs or liraglutide (10 nmol/kg) on (A) percent body weight change and (B) fat mass (FM)/lean mass (LM) loss in DIO mice following 10 days of treatment. Data are mean (SEM) for n=8 mice per treatment arm. *p <0.05, **p <0.01, ***p <0.001 compared to vehicle control animals.

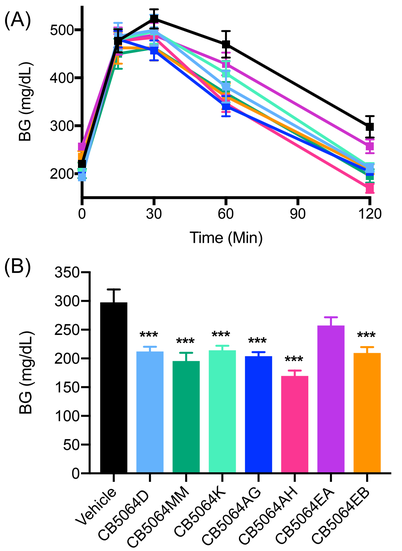


Figure 4: Glucose tolerance test in DIO mice following 10 days of treatment with CB5064 analogs. (A) Time course of blood glucose (BG) determined by glucometer after IP glucose bolus. (B) BG at 2 hours after glucose injection. Data are mean (SEM) for n=8 animals. ***p <0.001 compared to vehicle control animals. Note: food intake was not significantly affected by CB5064 analog treatment.

CONCLUSIONS

- Novel peptide analogs of CB5064 show agonist activity at the apelin/APJ receptor, stimulating APJ-mediated β-Arrestin recruitment and inhibiting cAMP production
- Several CB5064 analogs achieved maximum effects similar to the maximum effect of the natural substrate Apelin-13
- Active CB5064 peptides also produced significant body weight and fat mass loss and improved glucose tolerance in DIO mice after 10 days of treatment
- CB5064 analogs represent a novel class of peptides with potential for treatment of type 2 diabetes and other metabolic diseases

DISCLOSURES

Kent Grindstaff, Emily Stenger, Robin Shang, Tracy Yu, Wendy C. Luo, Virna V. Kim, Kenneth C. Cundy: Employees and shareholders of CohBar, Inc. Diego Perez-Tilve: Research funds from CohBar, Inc.