INTRODUCTION

Adrenocorticotropic hormone (ACTH), a peptide hormone that regulates glucocorticoid (GC) production by the adrenal gland through interaction with the melanocortin 2 receptor (MC2R), is involved in the regulation of the adrenal cortex. ACTH production by corticotropic cells in the anterior pituitary is regulated by corticotropin releasing hormone (CRH) and arginine vasopressin (AVP).

The secretion of ACTH is regulated by glucocorticoids. Chronic elevation of ACTH is associated with Cushings disease (CD) and Congenital Adrenal Hyperplasia (CAH). CD is characterized by the hypersecretion of ACTH by pituitary adenomas and loss of negative feedback control leading to chronic overproduction of cortisol which is ultimately responsible for the disease morbidity and mortality. The first line therapy, surgical removal of the pituitary tumor, suffers from high recurrence rates while pharmacotherapies are limited to insufficient efficacy or side effects. Therefore, there is a clear unmet need for new treatments (1-8).

In CAH, a loss of cortisol thus, reduced negative regulation of ACTH, results in ACTH dependent overproduction of adrenal androgens. The standard of care for CAH is treatment with GC. However, pharmacological levels of GC are needed to efficiently suppress androgens, leading to side effects (1,8).

Of the 5 melanocortin receptor subtypes, MC2R specifically interacts with ACTH and is selectively expressed in the adrenal gland. The hormone, a 39-residue peptide consisting of a common message sequence and one unique sequence (R), is the only known endogenous ligand for the MC2R (9,10). The receptor itself is a GPCR that stimulates cAMP production through coupling to Gs leading to expression of steroidogenic enzymes.

The interaction of ACTH and MC2R is proposed to occur through a multi-step mechanism whereby the activation of the gene encoding the receptor to promote a second interaction with the message sequence (10). We hypothesized that driving the potency and selectivity of the address-receptor interaction while disrupting the message-receptor interaction would lead to selective MC2R antagonists.

Such MC2R antagonists have the potential to efficiently regulate ACTH driven pathophysiology while avoiding the side effects of the current therapies in CD and CAH.

We report the discovery and pharmacological characterization of novel peptide MC2R antagonists in an optimized in vivo model of ACTH over secretion. Screening of rationally designed peptide libraries in functional assays allowed the identification of potent and selective peptide MC2R antagonists. These compounds suppressed ACTH induced MC2R signaling and displayed a significant reduction in corticosterone levels in primary human adrenal cortical cells. The optimized animal model of ACTH induced GC secretion was used to demonstrate the efficacy of our novel MC2R antagonists. These results demonstrate the potential of MC2R antagonists to address unmet needs in the treatment of CD and CAH patients.

REFERENCES


In vitro functional screening and selectivity assays

In vitro functional screening and selectivity assays were generated more than 100 rationally designed peptides based on the structural and conformational features of ACTH(1-39). Peptides were screened in vitro in cell based functional assays for agonist and antagonist activity at the MC2R and were counter-screened for agonist and antagonist activity against all other melanocortin receptors. Functional reduction of ACTH-induced cortisol production in primary human adrenal cortical cells was utilized for screening compounds with high potency and specificity. Data from MC2R and primary cell assays were utilized as starting points for peptide optimization.

In vitro pharmacodynamic model of hypercortisolism

To evaluate the in vivo efficacy of MC2R peptide antagonists, we developed a rat model of hypercortisolism by exogenously increasing plasma ACTH levels via continuous administration of ACTH(1-39) through a subcutaneous pump. ACTH levels were increased similar to the levels found in Cushings Disease patients (12).

In vivo reduction of cortisol in a hypercortisolism model

Dual (jugular vein and carotid artery) catheterized male Sprague-Dawley rats were implanted subcutaneously with Alzet® osmotic pumps administering 0.05 mg/kg/day ACTH(1-39) (Tocris) or vehicle for 7 or 14 days. Repeated exposure to ACTH leads to a reduction in body weight, primarily through reduced food intake (13). As expected, continuous infusion of ACTH(1-39) s.c. led to reduced body weight over 7 days and was utilized as a biomarker of elevated ACTH prior to inclusion in pharmacodynamics studies.

In vitro reduction of ACTH-induced cortisol production in primary human adrenal cortical cells

Adrenal cortical cells are capable of producing cortisol in response to stimulation with ACTH(1-39) (11). Functional activity of peptide compounds was measured by inhibition of ACTH-induced cortisol production in human adrenal cortical cells. Primary human adrenal cortical cells (HACD, Scientific) were cultured as previously described (11). Cells were treated for 24 hours with 100 ng/mL ACTH(1-39) (Tocris Bioscience) in the presence or absence of 1.2 µg MC2R antagonist peptides at 37°C. Following this incubation, conditioned media was collected and assayed for cortisol concentration by HTRF assay kit (Cisbio). In this single experiment, at the concentration used, both peptide 2 and peptide 4 reduced ACTH-stimulated cortisol production in primary human cells. Data are represented as mean +/- SD with 2 replicates per group.

CONCLUSION

We have identified selective and efficacious peptide MC2R antagonists in the low nM range by rational design and screening in functional cell based assays. In human primary adrenal cortical cells, MC2R peptide antagonists decreased ACTH-induced cortisol production. Utilizing an in vivo rodent model of hypercortisolism, we also demonstrated that MC2R peptide antagonists decreased elevated plasma cortisol without affecting increased plasma ACTH concentration.

These selective MC2R antagonists have the potential to generate lead structures towards the discovery of novel treatments for CAH and CD.