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Review

# Bench-top to clinical therapies: A review of melanocortin ligands from 1954 to 2016☆

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ABSTRACT

The discovery of the endogenous melanocortin agonists in the 1950s have resulted in sixty years of melanocortin ligand research. Early efforts involved truncations or select modifications of the naturally occurring agonists leading to the development of many potent and selective ligands. With the identification and cloning of the five known melanocortin receptors, many ligands were improved upon through bench-top *in vitro* assays. Optimization of select properties resulted in ligands adopted as clinical candidates. A summary of every melanocortin ligand is outside the scope of this review. Instead, this review will focus on the following topics: classic melanocortin ligands, selective ligands, small molecule (non-peptide) ligands, ligands with sex-specific effects, bivalent and multivalent ligands, and ligands advanced to clinical trials. Each topic area will be summarized with current references to update the melanocortin field on recent progress. This article is part of a Special Issue entitled: Melanocortin Receptors - edited by Ya-Xiong Tao.

## 1. Introduction

The melanocortin system consists of five receptor subtypes, discovered to date, that are involved in numerous biological pathways. The melanocortin-1-receptor (MC1R), expressed in the skin, is primarily involved in pigmentation [1,2]. The melanocortin-2 receptor (MC2R) is involved in steroidogenesis and is expressed in the adrenal cortex [2]. The centrally expressed melanocortin-3 and melanocortin-4 receptors (MC3R and MC4R) are linked to energy homeostasis [3–9]. Additionally, the MC4R has a role in sexual function in humans [10,11]. While the exact role of the melanocortin-5 receptor (MC5R) has not been elucidated [12,13], it has been linked to exocrine function [14].

A variety of endogenous ligands interact with the melanocortin receptors (MCRs). The naturally occurring agonists, derived from the proopiomelanocortin (POMC) gene transcript [15], stimulate the receptors to increase intracellular cAMP levels. Unique to this GPCR family is the presence of endogenous antagonists, agouti (ASP) and agouti-related protein (AGRP) [16–18]. Additionally, AGRP has been demonstrated to possess inverse agonist activity (directly decreasing cAMP levels within a cell) at the MC4R in mice and humans [19,20], while agouti has been shown to be an inverse agonist in cells expressing the grey squirrel MC1R [21].

Since changes in pigmentation can be readily visualized, early work on melanocortin ligands focused on the MC1R. The first reports of altered pigmentation dates back to 1916 [22,23]. Significant advances were achieved with the identification, sequencing, and cloning of the MCRs from 1992 to 1994 [1,2,6,7,9,12,13], coupled with the development of 96-well plate cAMP assays [24]. The genetic information combined with assay platforms generated an experimental paradigm that allowed for the design, synthesis, and investigation of potent, selective compounds for the different receptor subtypes. Many pharmaceutical companies initiated melanocortin ligand programs following the discoveries that the MC4R was linked to food intake, energy homeostasis, obesity, and sexual function in humans [5]. Reports of cardiovascular side effects associated with MC4R ligands [25] coupled with an increase in mergers within the pharmaceutical industry led to diminished industrial interest in melanocortin ligands. However, melanocortin ligands have continued to be advanced to clinical trials.

Over a century of work has been published on the melanocortin receptors, and 60 years of reports focused on melanocortin ligands have resulted in numerous discoveries. As there are too many ligands to summarize succinctly, the scope of this review will focus on the following topics. The first section will review select classic peptide melanocortin ligands followed by a summary of recent advancements in selective ligands. Next, a discussion of small molecule (non-peptide) ligands will focus primarily on the MC4R. Ligands resulting in sex-

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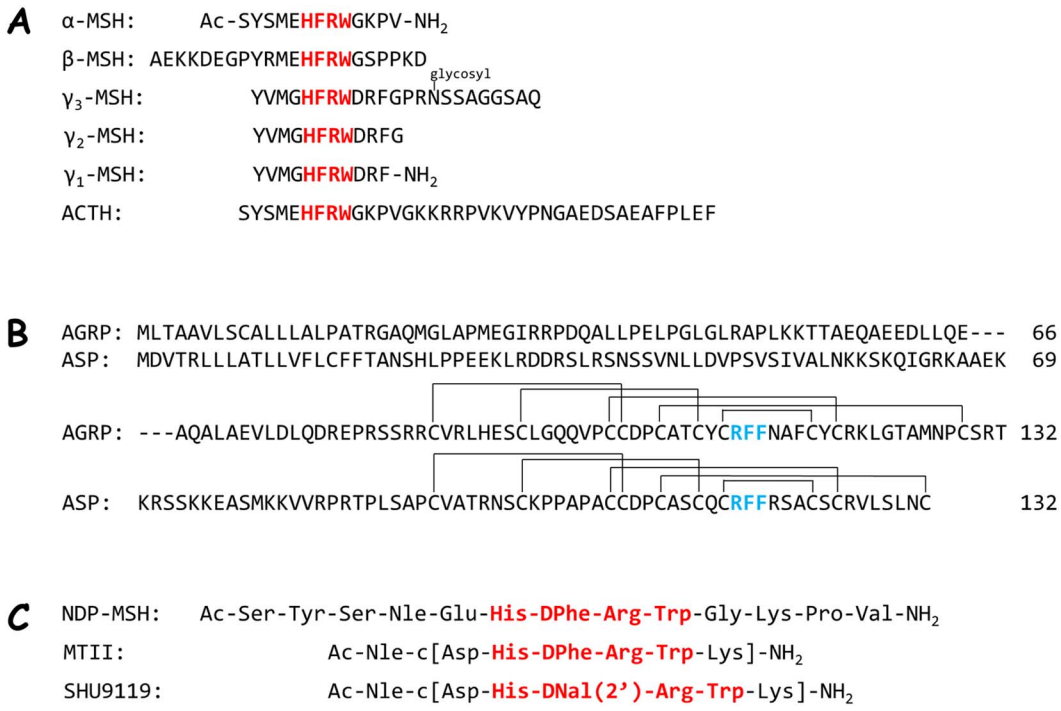


**Fig. 1.** Structures of classical melanocortin ligands. (A) POMC-derived naturally occurring agonists (the common His-Phe-Arg-Trp tetrapeptide is highlighted in red). (B) Sequences of the endogenous antagonists AGRP and ASP (the active Arg-Phe-Phe tripeptide is highlighted in blue). (C) NDP-MSH, MTII and SHU9119 (hypothesized pharmacophore region highlighted in red).

specific effects will be summarized, followed by an update of bivalent and multivalent ligands. A final section will highlight melanocortin ligands advanced to clinical trials, emphasizing compounds described between 2011 and 2016.

## 2. Classic peptide melanocortin ligands

Since the first reports of the sequences of adrenocorticotropic hormone (ACTH), β-melanocyte stimulating hormone (β-MSH), and α-MSH in the 1950s [26–28], numerous peptides and small molecule ligands have been developed for the MCRs. This section will focus on some author-perceived classic ligands. In particular, the naturally occurring ligands derived from the POMC gene transcript, the endogenous antagonists ASP and AGRP, and select synthetic derivatives of α-MSH (NDP-MSH, MTII, and SHU9119) will be highlighted (Fig. 1).

### 2.1. Proopiomelanocortin (POMC) gene transcript

The endogenous agonists for the melanocortin receptors are all derived from the POMC gene transcript [15]. Cleavage of the pre-proopiomelanocortin polypeptide sequence by prohormone convertases (PC) generates the melanocortin agonist ligands α-MSH, β-MSH, γ-MSH, and ACTH, as well as other peptides including β-endorphin and β-lipotropin [29–31]. Common to the endogenous melanocortin agonists is a His-Phe-Arg-Trp tetrapeptide sequence which is hypothesized to be the molecular recognition sequence for these ligands (Fig. 1A). This sequence is the minimally active truncation product that possesses agonist activity in the classic frog and lizard skin bioassays [32,33].

Since the endogenous agonists are derived from the POMC gene, the absence of the agonists in POMC-null individual has many effects on pigmentation (MC1R), steroidogenesis (MC2R), and weight gain (MC4R) [34–39]. Following the initial report of POMC-null humans, POMC knock-out (KO) mice were generated by removing the coding region for POMC derived peptides [40,41]. Similar to the phenotype observed in POMC-null humans, POMC KO mice experienced hyperphagia (MC4R), altered pigmentation (MC1R), and hypocortisolism (MC2R). While it was initially reported that adrenal glands were absent in POMC KO mice [40], it was subsequently observed that POMC mice possess adrenal glands that are significantly smaller than adrenal glands found in wildtype mice [41–43]. An intraperitoneal injection of an exogenous synthetic melanocortin ligand was able to alter the weight gain and pigmentation changes observed in these mice [40]. Untreated, the absence of the POMC gene is fatal in humans [36], underscoring the many critical functions these endogenous ligands perform *in vivo*.

#### 2.1.1. α-MSH

The α-MSH peptide is derived from the N-terminal 13 residues of ACTH (Fig. 1A) and is highly conserved across mammalian species. Both termini of α-MSH are modified, with the N-terminal acetylated and the C-terminal carboxyamidated (Fig. 1A). Acetylation of the N-terminal has been demonstrated to increase the stability of α-MSH compared to des-acetylated α-MSH [44,45]. The full length peptide possesses nonselective sub-nanomolar to nanomolar potencies at the MC1R, MC3R, MC4R, and MC5R [46,47]. Alanine scans of α-MSH have also indicated the importance of the $Met^4$, $Phe^7$, $Arg^8$, and $Trp^9$ positions for binding and functional activity at the mouse MC1R and rat MC3R [48,49]. A 2016 report examining the cloned mouse receptors indicated that in addition to positions $Met^4$, $Phe^7$, $Arg^8$, and $Trp^9$, the $Glu^5$ and $His^6$ positions also affect functional activity [47]. Expression of α-MSH in the central nervous system is predominantly in the hypothalamus [50]. Expression of α-MSH is dispersed throughout the arcuate nucleus, however it is found more densely in the lateral regions where it is synthesized [50,51]. Other locations of α-MSH expression include the dorsomedial nucleus of the hypothalamus (DMH), fibers in the medial preoptic, and the paraventricular, periventricular, and anterior hypothalamic nuclei [50,52]. The ability of α-MSH to decrease food intake in rodents following intracerebroventricular (icv) administration and alter the skin/hair coloration of humans and small mammals when dosed peripherally demonstrate the importance of this ligand in the regulation of several important pathways [53–59].