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Activation of MAPK by inverse agonists in six naturally occurring constitutively active mutant human melanocortin-4 receptors

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ABSTRACT

The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor that plays an essential role in regulating energy homeostasis. Defects in MC4R are the most common monogenic form of obesity, with about 170 distinct mutations identified in human. In addition to the conventional G_s-stimulated adenylyl cyclase pathway, it has been recently demonstrated that MC4R also activates mitogen-activated protein kinases, extracellular signal-regulated kinases 1 and 2 (ERK1/2). Herein, we investigated the potential of four MC4R ligands that are inverse agonists at the G_s-cAMP signaling pathway, including agouti-related peptide (AgRP), MCL0020, Ipsen 5i and ML00253764, to regulate ERK1/2 activation (pERK1/2) in wild type and six naturally occurring constitutively active mutant (CAM) MC4Rs. We showed that these four inverse agonists acted as agonists for the ERK1/2 signaling cascade in wild type and CAM MC4Rs. Three mutants (P230L, L250Q and F280L) had significantly increased pERK1/2 level upon stimulation with all four inverse agonists, with maximal induction ranging from 1.6 to 4.2-fold. D146N had significantly increased pERK1/2 level upon stimulation with AgRP, MCL0020 or ML00253764, but not Ipsen 5i. The pERK1/2 levels of H76R and S127L were significantly increased only upon stimulation with AgRP or MCL0020. In summary, our studies demonstrated for the first time that MC4R inverse agonists at the G_s-cAMP pathway could serve as agonists in the MAPK pathway. These results suggested that there were multiple activation states of MC4R with ligand-specific and/or mutant-specific conformations capable of differentially coupling the MC4R to distinct signaling pathways.

1. Introduction

G protein-coupled receptors (GPCRs) are versatile signaling molecules that regulate almost all physiological processes including energy homeostasis [1]. The melanocortin-4 receptor (MC4R) is a member of family A GPCRs that has been shown to be a critical regulator of energy homeostasis, regulating both energy intake and expenditure [2,3]. Human genetic studies have identified that defects in *MC4R* are the commonest monogenic form of obesity, characterized by its early-onset and severity [4]. About 170 *MC4R* mutations, including nonsense, missense, frameshift, and inframe deletions, have been identified from obese patient cohorts of different ethnic origins [5–7].

Since the cloning of the MC4R by Gantz et al. in 1993 [8], it has been established that it primarily couples to the stimulatory G protein (G_s), which increases adenylyl cyclase activity, and subsequently leads to increased cyclic AMP (cAMP) production that then enhances the activity of protein kinase A (PKA). The activation of MC4R by the endogenous agonist, α-melanocyte stimulating hormone (α-MSH), results in decreased food intake and increased energy expenditure, while the inhibition of MC4R by the endogenous antagonist, agouti-related protein (AgRP), leads to increased food intake [9,10].

Previous functional studies of MC4R on both naturally occurring and laboratory-generated mutants have relied primarily on measurements of intracellular cAMP generation. For example, studies on the naturally occurring mutations identified some mutations with defects in intracellular cAMP signaling (reviewed in [5–7,11]). However, some mutants show no functional alterations on intracellular cAMP accumulation [12–15], and some mutants even constitutively activate cAMP production [16,17]. Six naturally occurring mutations identified in obese patients, including H76R [15,18], S127L [14,19], D146N [15,18], P230L [14,19], L250Q [16,20,21] and F280L [15,22] (Fig. 1), have been shown to cause constitutive activation. The obesity observed in vivo in these patients could not easily be explained by the in vitro cellular phenotype of these mutations. Indeed, gain of function mutation is expected to result in lean phenotype, even anorexia nervosa. This suggests that signaling pathways other than G_s might contribute to the physiological effect of the MC4R. Indeed, in addition to the conventional G_s-cAMP–PKA pathway, it has been demonstrated recently that MC4R also activates p44/42 mitogen-activated protein kinases (MAPK), also known as extracellular signal-regulated kinases 1 and 2 (ERK1/2), both in vitro and in vivo in rat hypothalamus [23–25]. It has also been demonstrated that ERK1/2 is involved in melanocortin-induced decreases in food intake [26]. Thus, activation of ERK1/2 pathway is one cellular mechanism

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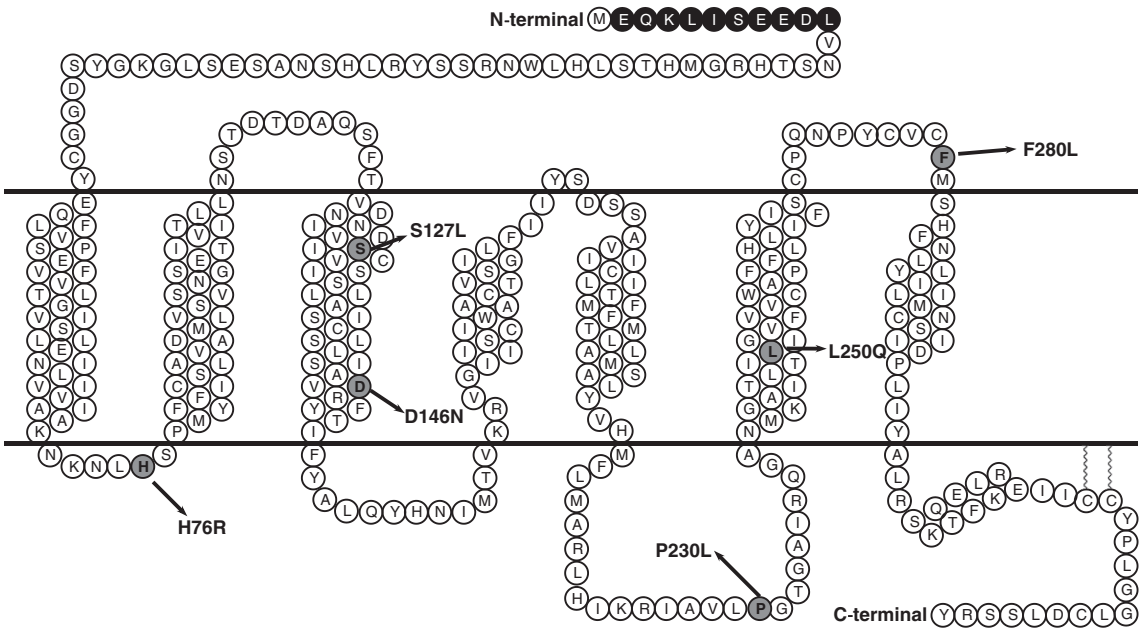

Fig. 1. Schematic model of the hMC4R. The naturally occurring constitutively active mutations characterized in this study are highlighted with gray background.

that may underlie the regulation of energy homeostasis mediated by the MC4R.

Recently, a few studies discovered the biased activation of ERK1/2 in MC4R. It has been shown that one mutant MC4R (D298N) retains cAMP generation but abolishes ERK1/2 activation [27]. We also reported that several artificially generated mutant MC4Rs have divergent cAMP and ERK1/2 signaling cascades upon agonist stimulation [28,29]. However, little is known about the alteration of ERK1/2 pathway in naturally occurring MC4R mutants, and how MC4R ligands may regulate the ERK1/2 signaling cascade that is crucial to gain a better understanding of MC4R functions at the molecular basis. In the present study, we assessed the potential of MC4R ligands, particularly antagonists, to regulate MAPK activation in wild type (WT) and the six naturally occurring constitutively active mutant (CAM) MC4Rs.

2. Materials and methods

2.1. Reagents and supplies

[Nle^4, D-Phe^7]-α-melanocyte stimulating hormone (NDP-α-MSH) and AgRP(83–132) (Fig. 2A) were purchased from Peptides International (Louisville, KY). Ac-D-2-Nal-Arg-2-Nal-NH_2 (MCL0020) [30] (Fig. 2B) was purchased from Tocris Bioscience (Ellisville, MO). Ipsen 5i [31] (Fig. 2C) and ML00253764 [32] (2-[2-[2-(5-bromo-2-methoxyphenyl)-ethyl]-3-fluorophenyl]-4,5-dihydro-1H-imidazol) (Fig. 2D)

A: AgRP (83-132)

Ser - Ser - Arg - Arg - Cys - Val - Arg - Leu - His - Glu - Ser - Cys - Leu - Gly - Gln - Gln - Val - Pro - Cys - Cys - Asp - Pro - Cys - Ala - Thr - Cys - Tyr - Cys - Arg - Phe - Phe - Asn - Ala - Phe - Cys - Tyr - Cys - Arg - Lys - Leu - Gly - Thr - Ala - Met - Asn - Pro - Cys - Ser - Arg - Thr - NH_2

B: MCL0020 **C: Ipsen 5i** **D: ML00253764**

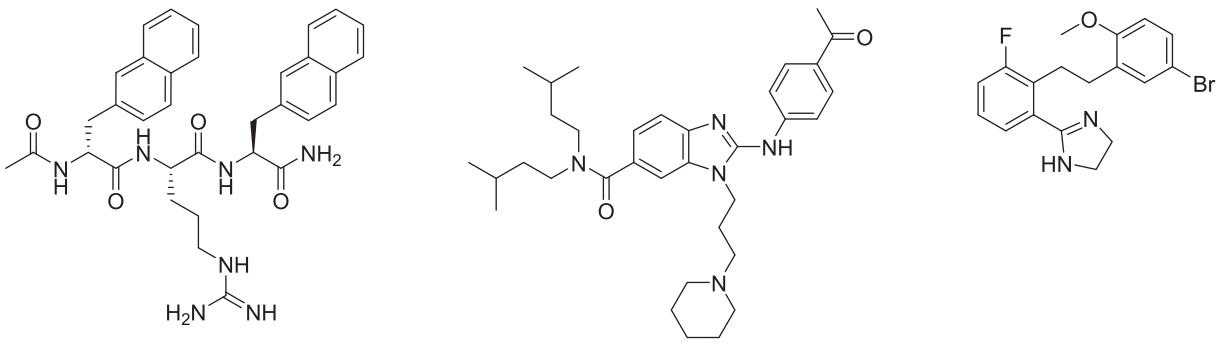

Fig. 2. Amino acid sequence of AgRP(83–132) (A) and chemical structures of MCL0020 (B), Ipsen 5i (C) and ML00253764 (D).