Topmouth culter (*Culter alburnus*) melanocortin-3 receptor: regulation of pharmacology by two isoforms of melanocortin receptor accessory protein 2

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ABSTRACT

Melanocortin-3 receptor (MC3R) is a regulator in energy homeostasis, and interaction of MC3R and melanocortin receptor accessory protein 2 (MRAP2) plays a critical role in MC3R signaling of vertebrates. However, the physiological roles of MC3R in teleosts are not well understood. Herein we cloned topmouth culter *mc3r* with a 984 bp open reading frame encoding a protein of 327 amino acids. qRT-PCR indicated that *mc3r* had higher expression in the central nervous system. In the periphery, *mc3r* was expressed in liver, testis and head kidney in the male, whereas it was mainly present in skin and ovary in the female. Culter MC3R expressed in HEK293T cells could bind to five peptide agonists with a higher B_{max} than human MC3R (hMC3R). In addition, caMC3R had higher affinity to ACTH and lower affinity to D-Trp^8-γ-MSH compared to hMC3R. All agonists could stimulate caMC3R and increase dose dependently intracellular cAMP accumulation. Culter MC3R showed a higher constitutive activity in cAMP signaling, higher efficacies and R_{max} to α-MSH, des-α-MSH, ACTH and D-Trp^8-γ-MSH. Culter MRAP2a remarkedly decreased cell surface expression but had no significant effect on total expression of caMC3R, whereas caMRAP2b had no effect on both cell surface and total expression of caMC3R. CaMRAP2a and caMRAP2b both remarkably decreased basal cAMP production. Furthermore, caMRAP2a significantly decreased B_{max} and R_{max}, while caMRAP2b did not affect ligand binding and agonist-stimulated cAMP. These results will promote further research on the physiological roles of MC3R in topmouth culter.

INTRODUCTION

The MC3R plays a vital role in regulating energy homeostasis as well as several other physiological functions.

MC3R is Family A G protein-coupled receptors (GPCRs) and it primarily couples to G protein (Gs) to activate adenylyl cyclase, leading to increased intracellular cyclic adenosine mono phosphate (cAMP).

Previous studies indicated that teleost MC3Rs show different pharmacological characteristics and physiological functions with mammalian MC3Rs.

MATERIALS AND METHODS

1. qRT-PCR was used to measure genes tissue distributions.
2. HEK293T cells were transiently transfected with hMC3R or caMC3R.
3. Ligand binding assays were performed using ^{125}I-NDP-MSH with or without different concentrations of unlabeled ligands.
4. Radioimmunoassay was used to detect cAMP levels.
5. Flow cytometry was used to determine receptors cell surface and total expression.

RESULTS

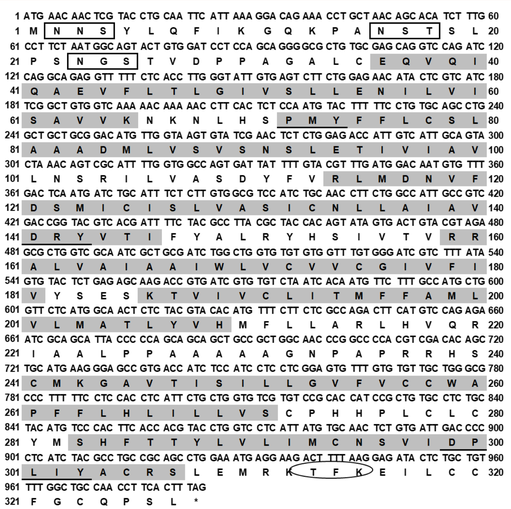

Fig. 1. Nucleotide and deduced amino acid sequences of caMC3R

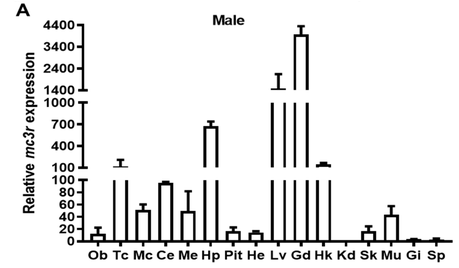

Fig. 2. Expression of culter *mc3r* in various tissues of (A) Male and (B) Female

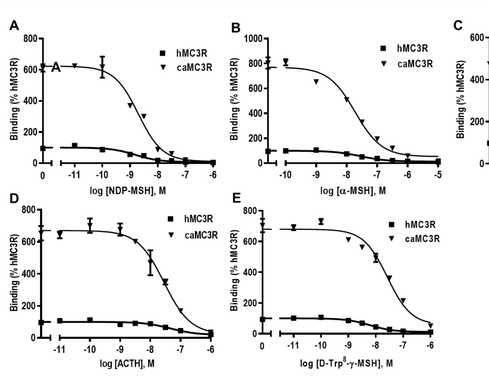

Fig. 3. Ligand binding properties of caMC3R

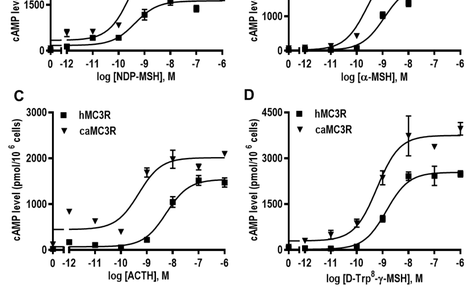

Fig. 4. Signaling properties of caMC3R

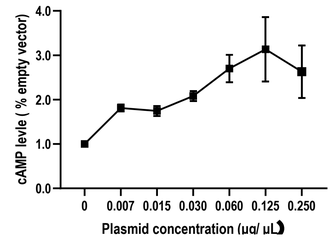

Fig. 5. Constitutive activities of caMC3R in cAMP signaling

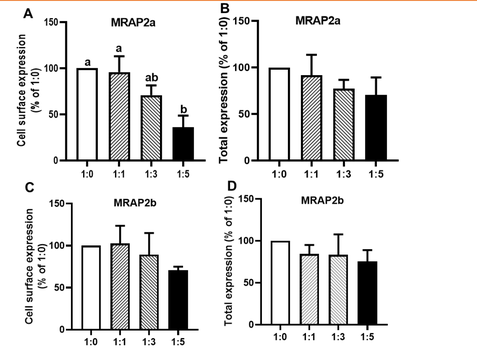

Fig.6. Expression of caMC3R regulated by MRAP2s

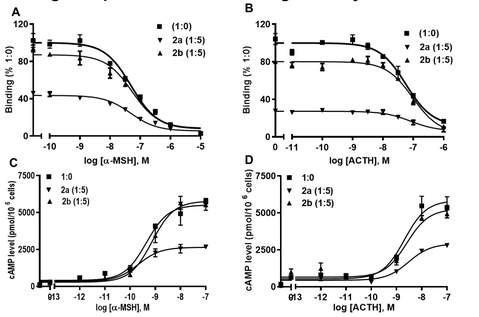

Fig.7. Modulation of caMC3R pharmacology by caMRAP2s

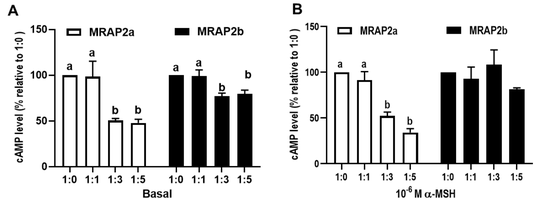

Fig. 8. The effect of caMRAP2s on caMC3R in cAMP signaling

CONCLUSIONS

1. Culter *mc3r* was primarily expressed in the central nervous system.
2. Culter MC3R had high constitutive activity.
3. Culter MRAP2a and MRAP2b had different effects on cell surface expression, ligand binding and cAMP production of caMC3R.