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REVIEW

Neuropeptide Y and melanocortin receptors in fish: regulators of energy homeostasis

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Abstract

Energy homeostasis, which refers to the physiological processes that the energy intake is exquisitely coordinated with energy expenditure, is critical for survival. Therefore, multiple and complex mechanisms have been involved in the regulation of energy homeostasis. The central melanocortin system plays an important role in modulating energy homeostasis. This system includes the orexigenic neurons, expressing neuropeptide Y/Agouti-related protein (NPY/AgRP), and the anorexigenic neurons expressing proopiomelanocortin (POMC). The downstream receptors of NPY, AgRP and post-translational products of POMC are G protein-coupled receptors (GPCRs). This review summarizes the compelling evidence demonstrating that NPY and melanocortin receptors are involved in energy homeostasis. Subsequently, the comparative studies on physiology and pharmacology of NPY and melanocortin receptors in humans, rodents and teleosts are summarized. Also, we provide a strategy demonstrating the potential application of the new ligands and/or specific variants of melanocortin system in aquaculture.

Keywords Energy homeostasis · Neuropeptide Y receptors · Melanocortin receptors · GPCRs · Fish (Teleost)

Introduction

G protein-coupled receptors (GPCRs) are ancient membrane proteins that exert highly conserved versatile signaling, with more than 1000 members identified with functional diversity (Bjarnadottir et al. 2006; Fredriksson et al. 2003; Tao 2020). Diverse extracellular signals, including light, odorants, ions, steroids, fatty acids, peptides and neurotransmitters, could act as the ligands of GPCRs (Bockaert and Pin 1999). After receptor activation by the ligands, the membrane-associated heterotrimeric G proteins serve as the molecular switches in GPCRs-regulated intracellular signal transduction pathways (Oldham and Hamm 2008). The heterotrimeric G proteins are composed of two functional units: an α subunit and a βγ dimer. In the absence of the ligands, the heterotrimeric G protein is inactivated, and the GDP-attached α subunit is tightly associated with the βγ dimer (Oldham and Hamm 2008). Binding of a signaling molecule to the receptor could activate the G protein with conformational changes, thus triggering the substitution of GTP for GDP on the α-subunit and the dissociation of α-subunit from βγ dimer (Tao et al. 2013).

The GTP-bound α-subunit could activate multiple signaling proteins, thereby regulating the intracellular signal transduction pathways via second messengers of cyclic AMP (cAMP), inositol 1,4,5-trisphosphate (IP3), diacylglycerol (DAG) and calcium (Ca^{2+}). Based on structural and functional similarity, the α-subunits could be broadly divided into four subtypes of stimulatory G protein ($G\alpha_s$), inhibitory G protein ($G\alpha_i$), $G\alpha_q$ and $G\alpha_{12/13}$ (Martemyanov and Garcia-Marcos 2018; Offermanns 2003). The $G\alpha_s$ protein could activate the adenylyl cyclase, resulting in increased cAMP concentrations, and subsequently activation of protein kinase A (PKA). $G\alpha_i$ inhibits the production of the cAMP. PKA could activate the functional proteins targeted in cytoplasm and the membrane, thus regulating the cellular functions via a faster non-genomic signaling. Also, PKA activates the proteins targeted in the nucleus [such as the cAMP response element-binding protein (CREB)] and modulates the cellular functions, via a slower genomic signaling (Yang and

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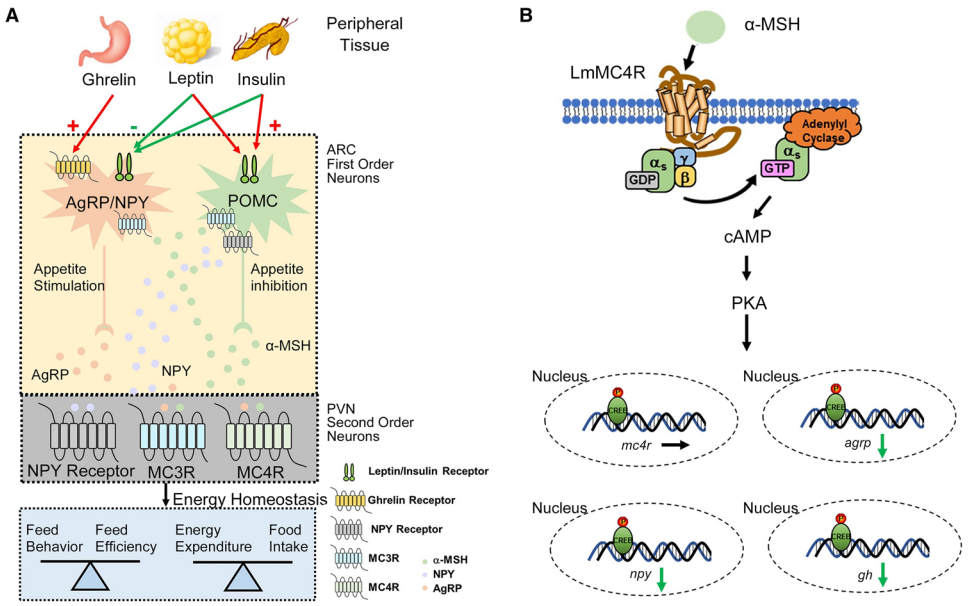

Fig. 1 Leptin-Melanocortin System. **A** Neurons expressing NPY/AgRP promote appetite-stimulating process whereas neurons expressing POMC lead to an appetite-suppressing process. In the hypothalamus, leptin activates the leptin receptors located in NPY/AgRP expressing neurons, and subsequently decreases appetite by suppressing AgRP/NPY. Leptin binds to the receptors on neurons expressing POMC, resulting in increased α-MSH. Energy homeostasis is regulated by neurons expressing POMC and AgRP/NPY and their cognate GPCRs. **B** In spotted sea bass, activation of MC4R signaling caused down-regulated *npy*, *agrp* and growth hormone gene (*gh*), suggesting that MC4R signaling activation could further inhibit the NPY/AgRP associated appetite-stimulating process and suppress growth

Yang 2016). For example, the phosphorylated CREB could transfer to the nucleus and then bind to the cAMP response element (CRE) in target genes, regulating gene expressions. Activation of $G\alpha_q$ could increase the intracellular IP3 and DAG via activating phospholipase C (PLC). IP3 activates the release of Ca^{2+} from the endoplasmic reticulum (ER). DAG and Ca^{2+} can activate protein kinase C (PKC), thus regulating enzyme activities, metabolic pathways and gene expressions. The $G\alpha_{12/13}$ subtype specifically target Rho guanine nucleotide exchange factors (RhoGEFs) and activates the RhoA kinase. In addition to the α-subunit-regulated second messengers signaling pathway, the βγ dimer and other proteins (such as β-arrestin) could also trigger the downstream signaling pathway and modulate cellular functions (Dorsam and Gutkind 2007; Miller and Lefkowitz 2001; Ritter and Hall 2009; Thomsen et al. 2016).

Energy homeostasis, which refers to biological processes that coordinate the energy intake and energy expenditure over a prolonged time, is important to maintain the long-term stability of the energy storage in organisms (Schwartz et al. 2000). Energy homeostasis not only regulates the normal energy expenditure, which is consumed by daily behavior and regular physiology, but also monitors the energy storage, which is essential for survival, development, growth and reproduction with low food availability (Schwartz et al. 2000). Despite daily energy intake may be variable, energy homeostasis enable animals to adjust cumulative energy intake in response to energy requirement changes (Morton et al. 2006).

Energy homeostasis is regulated by external factors, such as temperature, stress and food availability, as well as multiple internal factors, including genetic information, development stages, metabolite and hormone levels (Morton et al. 2006; Rønnestad et al. 2017). The hypothalamus is the hub that integrates these external and internal signals, thus regulating energy homeostasis (de Pedro et al. 2006). In the hypothalamus, the central melanocortin system plays an important role in regulating energy homeostasis (Tao 2010). This system includes the orexigenic neuropeptide Y/Agouti-related peptide (NPY/AgRP) expressing neurons and the anorexigenic neurons expressing proopiomelanocortin (POMC) in the arcuate nucleus, and the downstream receptors of melanocortin-3 and -4 receptors (MC3R and MC4R, Cone 2005). The post-translational products of POMC serve as agonists whereas the AgRP serves as an antagonist (or inverse agonist) to MC3R and MC4R (Tao 2010; Tao et al. 2013) (Fig. 1).

With more than 33,000 identified species, teleosts are the most diversified vertebrates. The large variations in genetics, ecology, morphology, anatomy and physiology of teleost species result in complex species-specific energy homeostasis regulatory mechanisms (Rønnestad et al. 2017). For example, a large proportion of teleosts continue to grow during the whole life span, which is in contrast with the determinate growth in mammals and model animals, such as zebrafish (*Danio rerio*) (Rønnestad et al. 2017). Moreover, the fish-specific genome duplication hypothesis, which hypothesizes that the duplication(s) of the whole genome

occurred in fish, indicates that fish genes/proteins have diverged significantly faster than mammalian homologues (Hoegg et al. 2004). In the context of the endocrine system, a larger number of endocrine receptor paralogs have been identified in fish (Boyce-Derricott et al. 2009). Therefore, neuroendocrine-regulated energy homeostasis need to be reviewed in comparison with mammals.

In recent decades, several studies have reported and/or reviewed the advances on the field of fish energy balance regulation. Some of these articles are general and represent comparative studies (Hoskins and Volkoff 2012), whereas some are focused particularly on a specific trait (Won and Borski 2013), a group of fish species (Demski 2012), or a single species (Matsuda et al. 2011). Volkoff (2016) and Rønnestad et al. (2017) published two outstanding review papers, and summarized systematically neuroendocrine and endocrine-regulated food intake (Rønnestad et al. 2017; Volkoff 2016). The purpose of this article is to provide a brief review of the physiology and pharmacology of the GPCRs in NPY- and melanocortin-regulated energy homeostasis in fish.

GPCRs in NPY- and melanocortin-regulated energy homeostasis

NPY receptor

The NPY family contains NPY, peptide YY (PYY) and pancreatic polypeptide (PP) (Holzer et al. 2012). The NPY is first isolated from porcine brain, and reported to be widely expressed in the central nervous system (CNS) with highly conserved protein sequences among mammals (Duarte-Neves et al. 2016; Larhammar et al. 2001; Tatemoto 1982). NPY plays a pivotal role in regulating food intake and energy homeostasis via activating their cognate GPCRs (reviewed in Lundell et al. 1995; Reichmann and Holzer 2016). In mammals, five functional NPY receptors (Y1, Y2, Y4, Y5 and y6) have been identified and these receptors are coupled with $G\alpha_i$ signaling (reviewed in Michel et al. 1998; Silva et al. 2005). The y6 receptor subtype is written with a small ''y'' because its physiological function has not been demonstrated. The mammalian Y1 subfamily contains Y1, Y4 and y6 subtypes, and these receptors share ~ 50% identities in amino acid sequences. The Y2 and Y5 subtypes are less conserved, showing only 30% homology to each other (Salaneck et al. 2001).

The fish NPY receptors were first identified from goldfish (*Carassius auratus*) and electric ray (*Torpedo marmorata*) (Blomqvist et al. 1992). Teleost NPY receptors are primarily expressed in brain and peripheral tissues, including eye and the gastrointestinal tract (Fredriksson et al. 2004; Lundell et al. 1997). The teleost NPY receptors could be further divided into six subtypes, including Y1 (zebrafish), Y2, Y4 (Ya), Y7, Y8a (Yc), and Y8b (Yb) receptors (Salaneck et al. 2008; Sundström et al. 2013). Previous studies in river lamprey (*Lampetra fluviatilis*) suggest that the Ya receptor is the fish ortholog of the mammalian Y4 receptor, whereas Yb and Yc seem to constitute a subtype not identified in mammals (Salaneck et al. 2001). Receptors of Y1 (zebrafish), Y4 (Ya), Y8a (Yc) and Y8b (Yb) are identified as the Y1 receptor subfamily; receptors of Y2 and Y7 belong to Y2 receptor subfamily (Larsson et al. 2005). The Y1 subtypes are identified widely in teleost species whereas the Y2 subtypes are found only in limited teleost species, such as zebrafish, rainbow trout (*Oncochynchus mykiss*), grouper and large yellow croaker *(Larimichthys crocea*) (Fredriksson et al. 2004; Larsson et al. 2006; Wang et al. 2014, 2019). With zebrafish, in addition to Y2 and Y7 receptors, the Y2 subfamily contains Y2-2 receptor, which is obtained by the local duplication of the Y2 receptor (Fällmar et al. 2011; Sundström et al. 2013).

Almost all teleosts produce NPY and PYY, whereas some teleost species further release PY (Cerdá-Reverter and Larhammar 2000). Consistent with previous results in mammals, NPY regulates food intake by mediating Y1 and Y5 receptors in teleosts (Larsson et al. 2005). In goldfish, food deprivation induces an up-regulated *npy* mRNA expression in the hypothalamus, and refeeding reverses the effects of food deprivation on hypothalamus *npy* mRNA expression (Narnaware and Peter 2001a; Narnaware et al. 2000). Intracerebroventricular (ICV) injection of Y1 or Y5 receptor agonists causes a dose-dependent effects on food intake in goldfish, and the increased food intake could be abrogated by NPY antagonists (de Pedro et al. 2000). Interestingly, the lower dosages of Y1 or Y5 receptor agonist could increase food intake, whereas higher dosages showed the opposite (de Pedro et al. 2000). The following studies showed co-administration of Y1 and Y5 receptor agonists resulted in an increased effect on food intake when compared with the individual administration of Y1 or Y5 receptor agonist (Narnaware and Peter 2001b). Moreover, suppression of one receptor failed to affect the responsiveness of the other one (Narnaware and Peter 2001b). These results probably indicate that Y1 and Y5 receptors independently regulate food intake in goldfish (Volkoff et al. 2005).

Physiology and pharmacology studies on NPY receptors are also reported in economically important teleost species. Food deprivation results in up-regulated hypothalamus *npy* mRNA expressions, and the ICV injection of NPY exhibits a dose-dependent increase in food intake in coho salmon (*Oncorhynchus kisutch*) and channel catfish (*Ictalurus punctatus*) (Silverstein and Plisetskaya 2000; Silverstein et al. 1998; Volkoff et al. 2005). The Yb receptor of rainbow trout has high affinity to human PP (Larhammar 1996), and the N-terminally truncated porcine NPY could bind to Yb

receptors in Atlantic cod and rainbow trout (Larson et al. 2003; Sharma et al. 1999). Because Y1 receptor subtypes exhibit conserved pharmacological characteristics in mammals and teleosts, some exogenous ligands, which are identified in human and mice studies, are probably involved in fish appetite regulation. These exogenous ligands, especially the small molecular agonist(s), should be tested further in aquaculture.

Functional studies of the teleost Y2 subfamily are limited. In goldfish, a previous study showed ICV injection of Y2 receptor agonist led to no effects on food intake (Narnaware and Peter 2001b). Despite zebrafish Y2-2 and Y2 receptor showing high sequence similarity, the Y2-2 receptor revealed reversed pharmacological characteristics with the Y2 receptor (Fällmar et al. 2011). Recently, two Y2 subfamily members (Y2 and Y7 receptor) were identified in large yellow croaker with different pharmacological characteristics. The endogenous NPY could activate both Y2 and Y7 receptors, whereas the truncated NPY activated only the Y2 receptor (Wang et al. 2019). Future studies should investigate the physiological functions of Y2 and Y7 receptors in regulating appetite of large yellow croaker via in vivo studies.

Melanocortin receptor

Melanocortins, including α-, β-, and γ-melanocyte stimulating hormones (α-, β-, and γ-MSH) and adrenocorticotropic hormone (ACTH), are produced by post-translational processing of POMC (reviewed in Dores and Lecaude 2005; Smith and Funder 1988). Melanocortins activate the melanocortin receptors (MCRs), thus regulating multiple physiological functions, including skin color, immunomodulation, steroidogenesis, energy balance and lipid metabolism (reviewed in Tao 2010). MCRs belong to family A rhodopsin-like GPCRs; five MCRs, named MC1R to MC5R, have been identified in mammals (Cone 2006). In these MCRs, the MC3R and MC4R are highly expressed in CNS with non-redundant roles in the regulation of energy homeostasis (Chen et al. 2000; Tao 2010). Activation of the MC3R and/or MC4R stimulates $G\alpha_s$ signaling, and enhances the intracellular cAMP accumulation (Rodrigues et al. 2015; Tao 2010). Melanocortins and other POMC-derived peptides are endogenous agonists; AgRP is the endogenous antagonist of MC3R and MC4R (Butler et al. 2017; Gantz and Fong 2003). In addition, analogs of α-MSH and some small molecules have also been identified as MC3R and/or MC4R ligands (Sawyer et al. 1980; Sebhat et al. 2002).

Melanocortin-3 receptor

In mammals, *MC3R* is widely expressed in brain regions and peripheral tissues, including the placenta, gut and immune cells of macrophages (Chhajlani 1996; Gantz et al. 1993; Jegou et al. 2000; Ni et al. 2006). The *mc3r* has been identified also in several teleost species, including zebrafish, spiny dogfish (*Squalus acanthias*) and channel catfish (Klovins et al. 2004; Logan et al. 2003b; Yang et al. 2019). However, based on genomic databases, the existences of *mc3r* orthologues in pufferfish species (*Tetraodon nigrov*iridis and *Fugu rubripes*) and spotted sea bass (*Lateolabrax maculatus*, unpublished data) have not been demonstrated (Logan et al. 2003a, b; Metz et al. 2006). The result that *mc3r* is absent in several teleost species is consistent with previous considerations that MC3R may serve as the receptor of γ-MSH, which is absent in teleosts (Arends et al. 1998; Kitahara et al. 1988).

Mouse genetic studies showed MC3R regulates feeding efficiency and fat storage, but does not regulate food intake (Butler et al. 2000; Chen et al. 2000). For example, *Mc3r* knockout mice did not exhibit hyperphagia and obesity when compared to the wild-type litter mates; these *Mc3r* knockout mice showed normal energy expenditure and even decreased food intake (Butler et al. 2000; Chen et al. 2000). However, the *Mc3r* knockout mice showed increased fat mass and reduced lean mass (Butler et al. 2000; Chen et al. 2000). Mice lacking both MC3R and MC4R exhibited exacerbated obesity when compared with MC3R or MC4R single gene knockout mice. This was further evidence that MC3R and MC4R have non-redundant functions in regulating energy homeostasis (Chen et al. 2000). In 2002, a potential loss-of-function *MC3R* mutation was identified from obese patients (Lee et al. 2002). The following study was convincing evidence that this mutant failed to convey ligand binding to $G\alpha_s$ signaling activation (Tao and Segaloff 2004). After that, several novel *MC3R* variants were identified from subjects, who had increased fat mass and decreased lean mass (Tao 2007). A recent study showed AgRP neurons expressing MC3R could exert inhibitory signaling to MC4R by modulating γ-aminobutyric acid (GABA) release onto anorexigenic MC4R neurons, thus regulating upper and lower boundaries of energy homeostasis (Ghamari-Langroudi et al. 2018).

Although MC3R is well studied in mammals, the studies on physiology and pharmacology of teleost MC3Rs are still limited. In rainbow trout, ICV injection of MC4R specific antagonist (HS024) is more potent than MC3R/MC4R antagonist (SHU9119) in increasing food intake (Schjolden et al. 2009). Based on previous studies in mammals, we may propose two hypotheses. First, rainbow trout MC3R plays a less important role in regulating appetite when compared to MC4R (Schjolden et al. 2009). Second, considering that MC3R modulates the anorexigenic MC4R signaling, MC3R antagonism probably alleviates the inhibitory signaling to MC4R (Ghamari-Langroudi et al. 2018). A recent study reported MC3R regulates the upper and lower boundaries of set point, or rheostasis during energy dyshomeostasis,

further supporting the second hypothesis (Ghamari-Langroudi et al. 2018). In channel catfish, MC3R exerts high constitutive activities in both cAMP and ERK1/2 signaling. The constitutive cAMP signaling could be suppressed by AgRP (Yang et al. 2019). Moreover, the melanocortin receptor accessory protein 2 (MRAP2) preferentially inhibited both the constitutive and agonist-triggered cAMP signaling rather than ERK1/2 signaling in channel catfish MC3R. It is likely that these results reveal a MC3R-regulated energy homeostasis network in teleosts (Yang et al. 2019).

Melanocortin-4 receptor

MC4R has been identified in several mammalian and non-mammalian species (reviewed in Tao 2010) with highly conserved amino acids sequences (Hughes et al. 2009; Staubert et al. 2007). In mammals, *MC4R* is primarily expressed in brain regions (Kishi et al. 2003; Mountjoy et al. 1994; van der Kraan et al. 1999), whereas teleost *mc4r* is expressed widely in both CNS and peripheral tissues, including gill, liver, intestine, and gonads (Zhang et al. 2019, 2020 and reviewed in Tao 2010). Due to teleost- or salmonid-specific whole genome duplication, teleosts exert increased *mc4r* paralogs. For example, four *mc4r* paralogs were identified in Atlantic salmon (*Salmo salar*) (Kalananthan et al. 2020).

Mice lacking *Mc4r* expression showed obesity and hyperinsulinemia with increased food intake and decreased energy expenditure (Huszar et al. 1997). Moreover, human genetic studies support the results observed in animal studies. In 1998, two groups independently identified frameshift *MC4R* mutations from patients with early-onset obesity showing that MC4R is important in regulating energy homeostasis (Vaisse et al. 1998; Yeo et al. 1998). After that, more than 175 distinct *MC4R* mutations associated with obesity and other diseases have been identified from patients (reviewed in Hinney et al. 2013; Tao 2009).

Also, MC4R plays an important role in regulating energy homeostasis in teleosts. Previous in vitro studies with MC4R ligands showed ICV injection of MC4R agonist (MTII, a superpotent analog of a-MSH) suppresses food intake whereas the MC4R antagonist (SHU9119) increases the food intake in rainbow trout and goldfish (Cerda-Reverter et al. 2003; Schjolden et al. 2009). In Mexican cavefish (*Astyanax mexicanus*), *mc4r* mutations contributed to physiological adaptations to nutrient-poor conditions by increasing appetite, growth, and starvation resistance (Aspiras et al. 2015). The short-term fasting resulted in down-regulation of *mc4r* with fluctuated changes in *agrp* and *npy* gene expressions in spotted sea bass. Conversely, down-regulated *mc4r* and *agrp* were observed in long-term fasting, showing that MC4R probably played a more important role in regulating long-term energy balance (Zhang et al. 2019). Likewise, the ya-fish (*Schizothorax prenanti*) showed up-regulated brain *mc4r* after short-term fasting (Wei et al. 2013).

Teleost MC4Rs show different pharmacological characteristics when compared to mammalian MC4Rs. [Nle^4, D-Phe^7]-α-MSH (NDP-MSH), which is a superpotent analog of α-MSH, is widely used in pharmacological studies of MCRs (Sawyer et al. 1980). Despite both human MC4R and teleost MC4Rs binding to NDP-MSH with high affinity, studies in spotted scat (*Scatophagus argus*) and several other teleost species consistently showed the maximal binding values of teleost MC4Rs were around 20–40% of that of the human MC4R (Li et al. 2017; Rao et al. 2019; Tao et al. 2020; Yi et al. 2018; Zhang et al. 2019). THIQ is a small molecule agonist in human MC4R. In teleosts, including spotted sea bass, swamp eel and spotted scat (*Scatophagus argus*), THIQ fails to displace NDP-MSH but stimulates intracellular cAMP accumulation, suggesting that THIQ acts as an allosteric agonist in teleost MC4Rs (Li et al. 2016; Yi et al. 2018; Zhang et al. 2019). A recent study with teleost MC4Rs showed that Ipsen 5i and ML00253764, which are two small molecule hMC4R antagonists (or inverse agonists), served as neutral allosteric modulators at cAMP signaling pathway but allosteric agonists at the ERK1/2 signaling pathway (Yang et al. 2020). Furthermore, this study showed that MCL0020, which is a peptidomimetic compound hMC4R blocker, exhibited divergent pharmacology on spotted scat (*Scatophagus argus*) and grass carp (*Ctenopharyngodon idella*) MC4Rs (Yang et al. 2020). The MCL0020 served as an inverse agonist for grass carp MC4R but a neutral antagonist for spotted scat MC4R (Yang et al. 2020).

Teleost MC4Rs show high constitutive activities in $G\alpha_s$ signaling (Li et al. 2017; Rao et al. 2019; Tao et al. 2020; Yang et al. 2020; Yi et al. 2018; Zhang et al. 2019). Human MC4R mutants with decreased constitutive activity are believed to be associated with obesity pathogenesis (Srinivasan et al. 2004; Tao 2005, 2008). However, in aquaculture, the fish with lower MC4R constitutive activity may exhibit a higher food efficiency, lower basal metabolism and faster weight gain. Development of molecular markers linked to MC4R genotypes with decreased constitutive activity probably contribute to selective breeding in aquaculture. In addition, inverse agonists, especially the small molecule compounds that suppress constitutive activity in teleost MC4Rs, may provide benefits for food intake promotion in aquaculture. For example, a recent study showed that MCL0020 acts as an inverse agonist for grass carp MC4R (Yang et al. 2020).

Future studies in aquaculture

Although it is controversial whether mutation in the *MC3R* is a cause for monogenic obesity, a large number of studies confirmed that *MC4R* is the most common monogenic

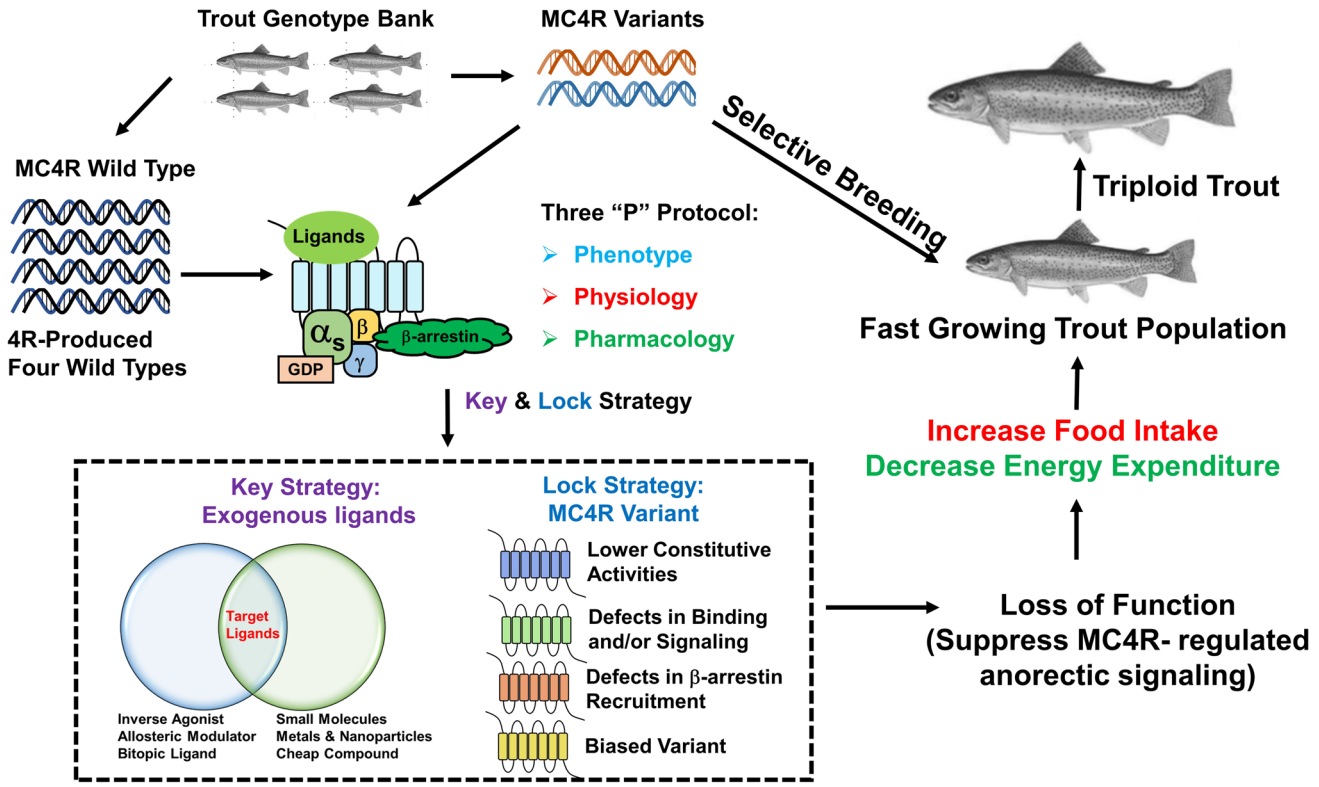

Fig. 2 A potential model for the application of the melanocortin system in aquaculture

form of obesity (Tao 2007). Therefore, variants of *MC3R* and *MC4R* are important targets for precision medicine and selective breeding (Kim et al. 2000; Lotta et al. 2019). For example, a recent study which was based on functional characterization over sixty *MC4R* variants identified in 500,000 people from UK Biobank, showed human gain-of-function *MC4R* variants with biased signaling could protect against obesity (Lotta et al. 2019). The relevance of the melanocortin system in the regulation of tenergy homeostasis has also been studied in agriculturally important species. In pigs, a *MC4R* mutation (D298N) was identified in certain strains with increased feed intake and growth rate (Kim et al. 2000). However, the subsequent pharmacological studies with this mutant showed divergent conclusions. One study showed this mutant failed to activate $G\alpha_s$ signaling (Kim et al. 2004), whereas another study indicated that D298N is functional (Fan et al. 2008). Studies of the melanocortin system in energy balance of economically important aquaculture species are still very limited.

Based on previous studies, we proposed a potential model for the application of the melanocortin system in aquaculture, with rainbow trout as an example (Fig. 2). The genome-wide (or genetic) association study may be used to investigate whether the *MC3R* and/or *MC4R* variant(s) are associated with economic traits, such as higher food intake, growth rate and/or weight gain. The three "P" protocols could be used to investigate the phenotype, physiology and pharmacology of trout carrying the variant. Subsequently, the "Key & Lock Strategy" could be used because the hormone and receptor interact with each other, like the key and lock. For Key Strategy, studies should be focused on identifying the exogenously low-cost ligands that decrease the high constitutive activities of trout MC4R. The potential targets include ligands that may serve as inverse agonists, (negative) allosteric modulator or bitopic ligands (ligands that concomitantly interact with the orthosteric and allosteric binding sites). The preferred ligands are non-peptide or small molecule compounds because they can be given by food rather than injection. In the Lock Strategy, the targets are the variants that exert lower constitutive activities and defects in binding and signaling. The purpose of the Key & Lock Strategy is to suppress the anorectic signaling of MC4R, thus increasing the food intake and decreasing the energy expenditure of cultured trout (Table 1).

Table 1 Comparison of selected parameters for tendons shortening scenarios Step-5a to Step-5c

Abbreviations	Full name	Abbreviations	Full name
ACTH	Adrenocorticotropic hormone	ICV	Intracerebroventricular
AgRP	Agouti-related peptide	MCRs	Melanocortin receptors
Ca^{2+}	calcium	MC3R and MC4R	Melanocortin-3 and melanocortin-4 receptors
CRE	cAMP response element	NPY	Neuropeptide Y
CREB	cAMP response element-binding protein	PYY	Peptide YY
CNS	Central nervous system	PY	Pancreatic polypeptide
cAMP	Cyclic AMP	PLC	Phospholipase C
DAG	Diacylglycerol		
ER	Endoplasmic reticulum	POMC	Proopiomelanocortin
Y1, Y2, Y4, Y5 or y6 receptor	Five functional NPY receptors, Y4 receptor (Ya receptor), Y8a receptor (Yc receptor), Y8b receptor (Yb receptor)	PKA	Protein kinase A
Ya receptor	Y4 receptor	PKC	Protein kinase C
Yc receptor	Y8a receptor	RhoGEFs	Rho guanine nucleotide exchange factors
Yb receptor	Y8b receptor	$G\alpha_s$	Stimulatory G protein
GPCRs	G protein-coupled receptors	NDP-MSH	[Nle^4, D-Phe^7]-α-MSH, a superpotent analog of α-MSH
$G\alpha_i$	Inhibitory G protein	α-, β-, and γ-MSH	α-, β-, And γ-melanocyte stimulating hormones
IP3	Inositol 1,4,5-trisphosphate	GABA	γ-Aminobutyric acid

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement Animal and human studies are not involved in this review paper.

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