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Review

Role of α-melanocyte stimulating hormone and melanocortin 4 receptor in brain inflammation

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ABSTRACT

Inflammatory processes contribute widely to the development of neurodegenerative diseases. The expression of many inflammatory mediators was found to be increased in central nervous system (CNS) disorders suggesting that these molecules are major contributors to neuronal damage. Melanocortins are neuropeptides that have been implicated in a wide range of physiological processes. The melanocortin alpha-melanocyte stimulating hormone (α-MSH) has pleiotropic functions and exerts potent anti-inflammatory actions by antagonizing the effects of pro-inflammatory cytokines and by decreasing important inflammatory mediators. Five subtypes of melanocortin receptors (MC1R-MC5R) have been identified. Of these, the MC4 receptor is expressed predominantly throughout the CNS. Evidence of effectiveness of selective MC4R agonists in modulating inflammatory processes and their low toxicity suggest that these molecules may be useful in the treatment of CNS disorders with an inflammatory component. This review describes the involvement of the MC4R in central anti-inflammatory effects of melanocortins and discusses the potential value of MC4R agonists for the treatment of inflammatory-related disorders.

Contents

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1. Introduction

Melanocortins are neuromodulatory peptides that share a seven amino acid core sequence and are generated by post-translational processing of the precursor protein pro-opiomelanocortin (POMC) [91]. The selective cleavage of POMC by prohormone convertases (PCs) yields alpha, beta and gamma melanocyte-stimulating hormones (α-MSH, β-MSH and γ-MSH) and adrenocorticotropin (ACTH). The activity of PCs, which belongs to the family of serine proteases, is tissue specific and their presence determines POMC selective expression [100]. The hormone α-MSH is a tridecapeptide that is produced in the presence of PC1, a convertase that cleaves POMC into ACTH and β-lipotropin, and PC2 that cleaves ACTH into α-MSH [4] (Fig. 1).

Melanocortins are expressed in a variety of tissues, but mainly in the pituitary gland and the central nervous system (CNS). Melanocortin-expressing neurons are found in the arcuate nucleus of the hypothalamus, and the nucleus of the solitary tract in the brain stem [29]. Melanocortin fibers project from these sites to the paraventricular nucleus, the lateral hypothalamus, and throughout the brain, e.g. amygdala, cortex, hippocampus, medulla, mesencephalon, and spinal cord [29].

The earliest known function of α-MSH was its ability to stimulate melanogenesis in melanocytes in the skin and therefore to increase pigmentation [69]. Later on, the cloning of five different melanocortin receptors allowed new research on the effects of α-MSH revealing its influence on energy homeostasis [17,54], stimulation of exocrine gland secretion [19], stimulation of erection [83], regulation of sexual behavior [118] and endocrine glands [27,66], and regulation of the cardiovascular system [53].

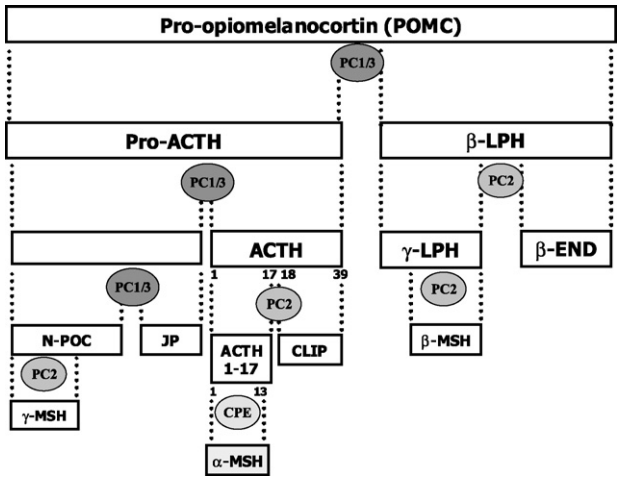

Fig. 1 – Diagram of POMC cleavage. The proprotein POMC is processed in a tissue specific manner. PC1 (also known as PC3) convertase cleaves this protein to generate pro-ACTH and β-lipotropin (β-LPH). Pro-ACTH is further cleaved by PC1 to generate ACTH, N-terminal peptide (N-POC) and joining peptide (JP). In the brain, PC2 cleaves ACTH into ACTH 1–17 and corticotropin-like intermediate lobe peptide (CLIP). Another peptidase (CPE) is needed to yield mature α-MSH from ACTH 1-17. PC2 also generates γ-lipotropin (γ-LPH) and β-endorphin (β-END) from β-LPH cleavage, β-MSH from γ-LPH, and γ-MSH from N-POC cleavage.

2. Melanocortin receptors

Five related G protein-coupled receptors mediate the actions of melanocortins: MC1R-MC5R. Each receptor is the product of a small, intronless separate gene. All MCRs have several N-glycosilation sites in their amino terminal domains, conserved cysteines residues in their carboxyl termini, and consensus recognition sites for protein kinase A (PKA) and C [124].

The MC1R was the first melanocortin receptor cloned from melanoma cells [20,88], and is known to play a pivotal role in the regulation of pigmentation in mammals. It is expressed primarily in melanocytes but it is also detected in immune cells including neutrophils, monocytes, dendritic cells, endothelial cells, and B lymphocytes [14], glioma cells, astrocytes [125] and in a few neurons of the periaqueductal gray [126]. The MC2R is the receptor for ACTH in the adrenal cortex [88] and its activation stimulates the secretion of adrenal steroids. MC2R expression was also detected in the skin [109], and in murine adipocytes [7]. MC3R is expressed in the CNS, the gastrointestinal system, and the kidneys [34]. In the CNS, the most intense expression was found in the ventromedial hypothalamus, medial habenula, ventral segmental and raphe areas. In the gastrointestinal system, this receptor is found in the stomach, duodenum and pancreas. The melanocortin effects mediated by MC3R are related to feeding and energy homeostasis [17]. Also, this receptor mediates the natriuretic effects of melanocortins, and among them, γ-MSH seems to be the most specific melanocortin in this respect [53].

MC4R is expressed primarily in the CNS [87,35] where it is the predominant MCR subtype and is detected widely in neuroendocrine and autonomic centers as well as in basal ganglia, hippocampus and cerebral cortex [63]. The most conspicuous sites where this receptor is expressed are the paraventricular nucleus in the hypothalamus and the dorsal motor nucleus of the vagus nerve [88,103]. Expression of functional MC4R in the hypothalamic GT1-1 cell line has also been detected [62]. The MC4R binds both α and β-MSH and, with lower affinity, γ-MSH. This receptor mediates melanocortin effects related to energy homeostasis and also erectile functions. Melanocortins acting on this receptor may also affect blood pressure and heart rate. Some synthetic agonists have proven to enhance sexual functions and erectile activity in males and to increase sexual desire and genital arousal in females [44].

MC5R is expressed in exocrine glands, but not in the CNS [64]. The ligand with the highest binding affinity to this receptor was shown to be α-MSH. This receptor is mostly expressed in sebaceous, Harderian, lacrimal, and preputial glands. Likely, this receptor mediates the stimulation of the production of pheromones by the preputial glands, therefore influencing sexual behavior [9].

Knock-out mice models and genetic studies have pointed to the importance of the melanocortins in complex human pathways such as pigmentation, lipolysis, food intake, thermo-

Table 1 – Ligands and functions of melanocortin receptors

Receptor subtype	Ligand affinity	Physiological functions
MC1R	α-MSH = β-MSH = ACTH > γ-MSH	Pigmentation, inflammation
MC2R	ACTH	Steroidogenesis
MC3R	γ-MSH = β-MSH = ACTH = α-MSH	Energy homeostasis, natriuretic activity, inflammation
MC4R	α-MSH = β-MSH = ACTH >> γ-MSH	Energy homeostasis, erectile activity, sexual behavior, inflammation and neuroprotection
MC5R	α-MSH ≥ β-MSH = ACTH > γ-MSH	Exocrine secretion

genesis, sexual behavior, memory and inflammatory responses. MC3R and MC4R are likely targets for controlling body weight; MC1R may be used in the treatment of inflammation and MC2R for the treatment of glucocortical deficiency. A role for MC5R still remains unclear, but the evidence suggests a role in exocrine gland function [19] (Table 1).

There are many complex and unique aspects of melanocortin signaling such as the existence of endogenous antagonists, the agouti proteins that act at three of the five melanocortin receptors. The agouti protein is produced in the skin and binds MC1R whereas the agouti-related protein (AgRP) is present in the brain where it promotes increased feeding and decreased energy expenditure by binding MC4R [22]. The system is also unique from a regulatory point of view as it is composed of fibers expressing both agonists and antagonists of melanocortin receptors. In contrast to many peptides, the melanocortin agonists and antagonists are expressed in a limited number of very discrete locations. Similarly, the melanocortin receptors are also expressed in a limited number of discrete locations where they tend to be involved in rather circumscribed physiological functions [22]. In the CNS, melanocortin peptides are agonists of the MC3R and MC4R, whereas AgRP is a high-affinity antagonist of both these receptors. AgRP and α-MSH are believed to be the natural antagonist and agonist respectively of MC3R and MC4R.

MC4R has generated wide interest for its involvement in obesity. Pharmacological [30,23] and genetic [54] studies demonstrate that this receptor is critical for the regulation of energy homeostasis. Furthermore, haploinsufficiency of the MC4R is linked to obesity in up to 4% of severe cases in humans [31,117]. The central administration of melanocortins is capable of regulating both feeding [30] and metabolism [23,47]. Therefore, MC4R is a potential target for new antiobesity drugs.

The expression of the MC4R is regulated by stress exposure. Several reports have indicated that the stimulation of the MC4R activates the hypothalamus–pituitary–adrenal (HPA) axis, and that the MC4R mediates stress-related behaviors and anxiety in rodents [122]. The recent development of selective antagonists for MC4R has provided pharmacological evidence that the blockade of MC4R could be a useful way of alleviating numerous conditions such as anxiety, depression and addiction to drugs of abuse [16].

3. α-MSH and inflammation

α-MSH is normally produced in the pituitary, brain, and several peripheral tissues including immune cells, and has been shown to play a crucial role in the regulation of immune and inflammatory reactions. Anti-inflammatory effects of α-MSH can be elicited through centrally expressed MCRs, which orchestrate descending neurogenic anti-inflammatory pathways. On the other hand, α-MSH can also exert anti-inflammatory effects on the cells of the immune system as well as on resident non-immune cell types of peripheral tissues.

Several findings indicate that α-MSH inhibits the production and activity of pro-inflammatory cytokines while enhancing the secretion of anti-inflammatory cytokines. α-MSH has been shown to interact with various cells of the immune system and to downregulate either the production or the action of the pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, IL-2, and IFN-γ [71,110,76], and thus acting as an anti-inflammatory agent. In macrophages, α-MSH decreases nitric oxide (NO) production by inhibiting the NO synthase [110]. In contrast, the synthesis of the anti-inflammatory cytokine IL-10 is up-regulated by α-MSH [5].

Endotoxins, like lipopolisaccharide (LPS), are surface bacterial polysaccharides that can elicit an immune response. α-MSH can antagonize many of the biological effects of LPS and of the pro-inflammatory cytokines, including effects on body temperature, immune and endocrine functions, and behavior [12]. α-MSH has been shown to act directly on MCRs on peripheral immune cells to down-regulate pro-inflammatory cytokine production in response to endotoxin in vitro [112]. The intracerebroventricular (icv) injection of α-MSH has also been shown to inhibit peripheral inflammation, such as in the skin [72]. This appears to be mediated by descending anti-inflammatory neural pathways induced by the stimulation of MCRs within the brain [79]. Central MCRs have also been

shown to modulate temperature, neuroendocrine, and behavioral responses to inflammatory stimuli [108,127,81].

The expression of MC1, 3, and 5 receptors has been reported in immune cells. MCRs have been detected in rodents on peritoneal macrophages and splenic lymphocytes and on circulating human monocytes and macrophages [112,36,93,1]. There are data supporting a functional role for both MC1R and MC3R in modulating inflammatory responses [112,36]. Experimental data support the notion that agonist activity at MC3R can be used for the design of novel drugs to treat inflammatory conditions [37]. However, the central anti-inflammatory actions of α-MSH seems to be mediated by MC4R [10,11].

α-MSH participates in the physiological regulation of the pyretic and HPA responses to inflammation [98]. Icv infusions of α-MSH attenuated the HPA response to IL-1β, whereas the α-MSH antagonist, AgRP, enhanced this effect, suggesting that endogenous α-MSH plays a physiological role in this process [127]. α-MSH may act both centrally and peripherally to modulate the HPA response to inflammatory stimuli. In monkeys, the intravenous administration of α-MSH agonist [Nle4, d-Phe7]α-MSH (NDP-α-MSH) attenuated the release of IL-1β, TNF-α, and IL-6, which are known to stimulate the HPA axis [116]. On the other hand, icv infusion of α-MSH attenuated the HPA response to icv IL-1β in the monkey, consistent with an effect on central MCRs [106]. Moreover, in rodents, α-MSH inhibits CRH release from the hypothalamus [77,129].

4. Central anti-inflammatory actions of α-MSH

It has been known for over 20 years that the central administration of α-MSH can suppress fever [41,114]. α-MSH serum levels increase during endotoxemia [82], after administration of pyrogens and in inflammatory conditions [13]. The MC4R appears to be the mediator of the antipyretic effects of α-MSH since it appears to participate in regulation of thermoregulatory responses, including fever. Anatomic studies have revealed the presence of MC4R mRNA-expressing cells and high densities of specific melanocortin-binding sites in preoptic, hypothalamic and brainstem nuclei implicated in thermoregulation and fever [63,113]. Moreover, the suppression of LPS-induced fever by icv α-MSH was prevented by an equimolar coinjected dose of the MC4R-selective antagonist HS014 [108]. On the other hand, centrally administered α-MSH potentiates fever in the presence of MC4R blockade. This finding may be relevant to recent findings implicating an important role of central MCR subtypes in mediating the anorexia and cachexia associated with inflammation and chronic illness [67].

Novel small molecule MC4R agonists such as, Ro 27-3225 and [1R-(4-chloro-benzyl)-2-(4-cyclohexyl-4-[1,2,3,]triazol-1-ylmethyl-piperidin-1-yl)-2-oxo-ethyl]-amide (THIQ) have been considered ideal anti-obesity drugs due to their low molecular weight, enzymatic stability and potential selectivity for MC4R versus MC3R. Few studies have examined the role of these compounds in inflammation. Ro 27-3225 improves survival after haemorrhagic shock and shows anti-shock effects inhibiting free radicals formation through activation of MC4R located within the brain [39]. THIQ, administered together with LPS, possesses anti-inflammatory activity and to some extent inhibits the LPS-induced increase in NO levels in a mouse model for brain inflammation [89].

We have observed that melanocortins inhibit NO and prostaglandin (PG) production induced by IL-1β in the hypothalamus [24] and that icv administration of α-MSH attenuates the hypothalamic expression of iNOS and COX-2 during endotoxemia by activating MC4R [11]. These findings suggest that α-MSH anti-inflammatory effects involve down-regulation of iNOS and COX-2 expression, reducing inflammatory mediators such as NO and PGs which have been associated with pro-inflammatory activities in neurodegenerative processes of several acute and chronic brain diseases [90,84].

It was pointed out that MC1R and MC3R are involved in the anti-inflammatory effects of melanocortins in the periphery [78,65] but MC4R is the primary candidate for the central anti-inflammatory action of melanocortins [11,56]. Moreover, a recent study reported that the selective MC3R agonist, γ-MSH, had no protective effect in the ischemic stroke, whereas a MC4R agonist, NDP-α-MSH, did exert a potent neuroprotective effect [38].

Melanocortins have a life-saving effect in animal and human hypoxic conditions such as circulatory shock, prolonged respiratory arrest and myocardial ischemia [3,39,42,43,75]. The treatment with α-MSH decreases the expression of TNF-α and IL-1β after brain ischemia in dogs [51] and mice [52]. Moreover, decreased α-MSH plasma levels have been detected in patients with acute traumatic brain injury and decreased levels have been recorded in patients with unfavorable outcome [13].

The α-MSH agonist, NDP-α-MSH, has been shown to exert a strong neuroprotection, through the activation of MC4R, against damage consequent to transient global cerebral ischemia in gerbils [38] and in focal cerebral ischemia induced by endothelin-1 in rats [40]. An ischemic brain injury in rats was found to increase mRNA expression of MC4R (but not MC3R or MC5R) in the controlateral, uninjured striatum [86]. Accordingly, Hwang et al. [55] reported that global cerebral ischemia induced an ectopic expression of ACTH-like immunoreactivity in the gerbil hippocampus. Consistent with the idea that the protective effect of melanocortins against ischemic stroke occurs through selective stimulation of MC4R, administration of γ2-MSH, a selective agonist of MC3R, did not reduce cortical and striatal damage in two rat models of focal cerebral ischemia [48]. Some studies also suggest that α-MSH promotes restoration of injured nerves and the spinal cord [59]. Taken together, these data indicate that melanocortins might be physiologically involved in neuroprotection through the activation of central MC4R.

5. Effect of α-MSH on glial cells

The anti-inflammatory actions of melanocortins could be exerted through different routes including central activation of MCRs on inflammatory cells that would lead to a local control of inflammation within the brain. Indirect effects mediated by melanocortins, such as diminished death signals from non-neuronal cells, e.g., astrocytes, cannot be ruled out.

α-MSH is known to cross the blood–brain barrier [2] and the efficacy of intraperitoneal NDP-α-MSH treatment preventing cerebral ischemia indicates that it has sufficient access to MC4R-expressing cells either within the brain or in systemically exposed elements of the neurovascular unit. Previous studies reported widespread expression of MC4R in low levels as well as dense expression within specific neuron-rich areas such as the hypothalamus and the hippocampus [74,63]. The widespread low-level expression of MC4R may be indicative of their presence in non-neuronal cells. Indeed, a significant astrocyte hyperplasia was found inside ischemic areas of NDP-α-MSH-treated animals [40].

Human microglial cells express several MCR subtypes (MCR 1, 3, 4 and 5) [70]. MC1R is expressed in astrocytes but it was reported that this receptor is not involved in the anti-inflammatory effects of α-MSH [56]. Selkirk et al. [105] reported the selective expression of MC4R with analysis of mRNA levels and established a functional response of MC4R using cAMP accumulation in cultured rat astrocytes. Accordingly, we reported that rat astrocytes express MC4R, as we detected MC4R mRNA and protein expression. On the other hand we showed no expression of MC3R in these cells [10].

α-MSH and its COOH-terminal tripeptide (KPV) inhibited TNF-α production induced by a bacterial endotoxin in cells of a human glioma line [125]. Melanocortins suppress the transcription factor NF-κB activation and expression of TNF-α and iNOS in activated microglia and peripheral macrophages [13,26,33] and also inhibited iNOS and COX-2 induction within rat hypothalamus in vivo through MC4R [11]. α-MSH acting on MC4R attenuates the increase of NO and PGE2 release and iNOS and COX-2 expression induced by LPS/IFN-γ in astrocytes [10], suggesting that the anti-inflammatory actions of α-MSH in the brain may result, in part, from a direct action on these glial cells.

The neuroprotective effect of melanocortins could be the consequence of direct modulation of mechanisms such as inflammatory reaction and apoptosis associated with neuroinflammatory diseases. Recent reports indicate that α-MSH has an antiapoptotic role in different cell types [6,49,68] and in the ischemic renal failure [58]. These studies suggested that α-MSH has a protective effect on melanocytes and tubular renal cells by modulating Bcl-2 protein levels [68]. Reactive astrocytes have been implicated in the pathology of neuroimmunological diseases such as multiple sclerosis, ischemia and Alzheimer disease. Reactive astrocytes produce NO and pro-inflammatory cytokines and chemokines [28]. Thus the control in the activation of astrocytes can be effective in decreasing the severity of neurodegenerative diseases. α-MSH, acting on MC4R, prevented apoptosis of rat astrocytes by blocking the increase of the Bax/Bcl-2 ratio induced by LPS plus interferon-γ and α-MSH per se increased Bcl-2 levels [10]. The antiapoptotic action of α-MSH could be important at early stages of the inflammatory response when preservation of astroglial function is necessary to promote neuron survival. These results are consistent with the antiapoptotic action of α-MSH via MC4R in brain ischemia [38] and in neuronal death induced by excitotoxicity in the hippocampus [32]. MC4R up-regulates an endogenous neuroprotective pathway enhancing two anti-apoptotic enzymes Bcl-2 and Bcl-xL in hippocampal neurons [38].

6. α-MSH and autoimmunity

α-MSH induces regulatory T (T reg) cells that are antigen-specific and require the presence of the antigen in a target tissue to mediate immunosuppression. It was reported that it is possible to generate antigen-specific T reg cells by α-MSH in vitro and then transfer them intravenously to suppress antigen-specific T helper type I cells mediating inflammation [92,96]. This finding opens the possibility to use this melanocortin to induce antigen-specific T reg cells to prevent and suppress autoimmune diseases. In fact, in vitro generated α-MSH-induced T reg cells suppressed ocular autoimmune disease in vivo [95]. It has been reported that α-MSH may be useful as treatment of inflammatory experimental autoimmune encephalomyelitis [128]. This is a T-cell mediated inflammatory autoimmune process of the CNS that resembles in some aspects the human demyelinating disease multiple sclerosis. Orally administered α-MSH can reduce the signs of the disease and inhibit CNS inflammation [8]. Moreover, Han et al. [46] proposed that genetic engineering of self-reactive T cells with α-MSH may represent a clinically viable approach to the treatment of autoimmune diseases. The use of α-MSH in gene therapy would be a desirable new approach to the prevention and treatment of autoimmune diseases such as multiple sclerosis that results in a neurological impairment.

7. α-MSH mechanisms of action

Although the anti-inflammatory effects of melanocortin peptides have been clearly demonstrated, their mechanisms of action are not well understood. Several studies have addressed the molecular mechanisms by which α-MSH may exert its anti-inflammatory effects. α-MSH has been shown to block LPS receptor signaling, on macrophages and to attenuate endotoxin stimulation of macrophages [115]. In addition, α-MSH has been reported to inhibit the production of chemokines, endothelial cell adhesion molecules, PGs and NO, which all contribute to the inflammatory process [104,110]. Thus, it seems that α-MSH can affect the inflammatory response at multiple levels and by multiple mechanisms.

The transcription factor NF-κB is required to induce expression of inflammatory cytokine genes in response to various inflammatory agents. There are large numbers of genes involved in cellular inflammation that need NF-κB activation, including TNF-α, IL-1, IL-6, chemokines, cyclo-oxygenase, lipoxygenase, cell adhesion proteins, and NO synthase [107]. α-MSH has been shown to block the activation of NF-κB in several experimental models [56,57,80,102]. For example, α-MSH suppresses nuclear translocation of NF-κB induced by TNF-α and this effect could be mediated by an intracellular increase of cAMP [80]. α-MSH has been shown to inhibit NO and TNF-α production [80,102, 110] and PG synthesis [73], which are NF-κB-dependent and are involved in inflammation. In addition, the p65 subunit of NF-κB has been colocalized with α-MSH in the rat brain, suggesting a close relationship [60].

Thus, the anti-inflammatory properties assigned to α-MSH are due to its ability to down-regulate the activation of NF-κB by a variety of inflammatory stimuli in cells of the immune

system. Since α-MSH has no known pharmacologic toxicity and is able to suppress NF-κB, it has potential for use in conditions initiated through NF-κB activation, such as inflammatory diseases, HIV replication in AIDS, and septic shock.

This suppression of NF-κB activation by α-MSH was not cell type specific but it was mediated through generation of cAMP and activation of PKA [80]. Reports show that elevation of cAMP reduces NF-κB activity [18,50,94,97]. Ollivier et al. [97] found inhibition of NF-κB-mediated transcription by elevated cAMP or by overexpression of PKA without any inhibition of the IκBα degradation or nuclear translocation of p65. In contrast, Chen and Rothenberg [18] and Neumann et al. [94] reported that the effects of cAMP are mediated through stabilization of IκBα and impairment of the nuclear transport of p65. Similarly, Manna et al. [80] found that the elevation of intracellular cAMP induced by α-MSH inhibited IκBα degradation and p65 translocation to the nucleus as well as gene transcription. It was also reported that the catalytic subunit of PKA binds IκBα, the inhibitory subunit of NF-κB in the cytoplasm [130].

Ligand binding to MCRs activated adenyl cyclase, which led to the production of cAMP and subsequent activation of a cAMP response element-binding protein (CREB) [85]. α-MSH had an activating effect on hypophysiotropic TRH neurons via the phosphorylation of CREB [66] and MC4R agonist increased phosphorylation of both ERK1/2 and CREB in the solitary nucleus of the rat [106]. CREB is responsible for cell survival during episodes of metabolic or oxidative stress [99] and

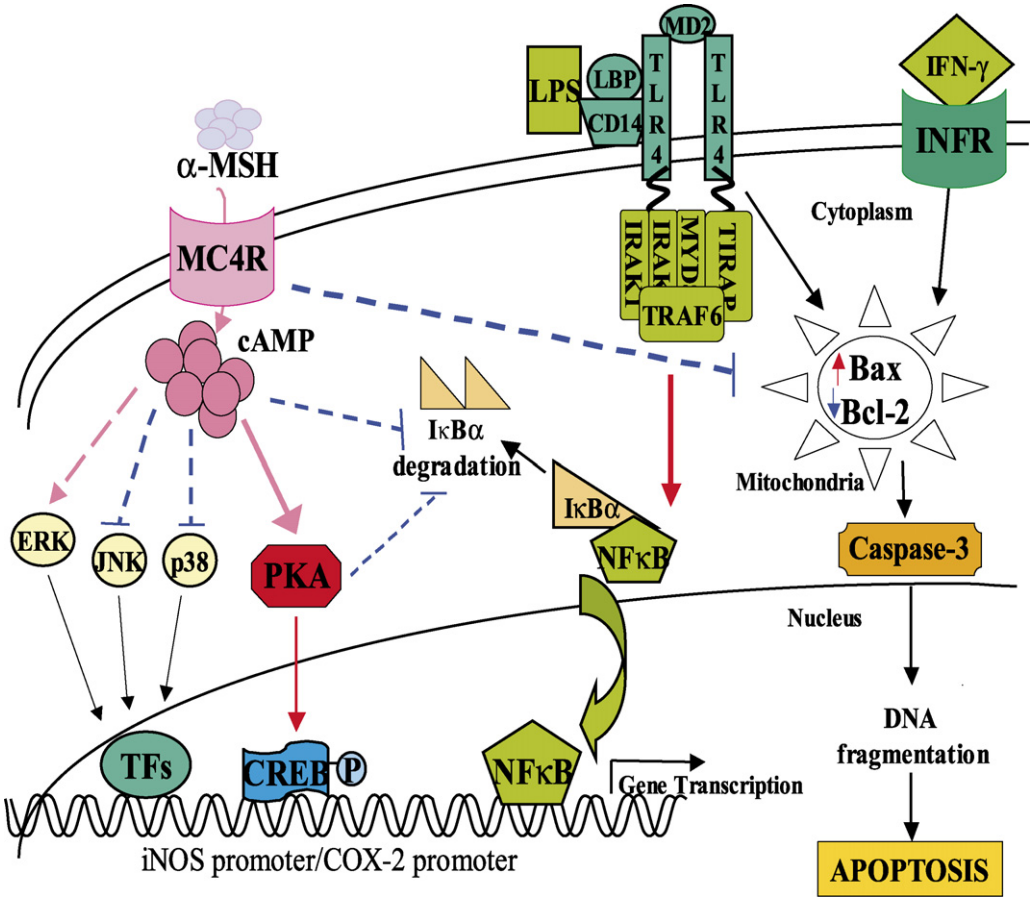

Fig. 2 – Model for MC4R signaling. α-MSH binds to and stimulates MC4R leading to the production of cyclic AMP (cAMP). Then, cAMP will activate protein kinase A (PKA) leading to the phosphorilation of the cAMP-responsive element-binding protein (CREB). The activation of CREB induces its binding to cAMP-responsive element (CRE) sequence in the target genes. Target genes expression could be induced by pro-inflammatory stimuli like bacterial lipopolisaccharide (LPS). LPS is helped by CD14 to bind its receptor (TLR4). LPS signaling induces the recruitment of several adaptor proteins leading to the degradation of the inhibitor (IκBα) of the nuclear factor-κB (NF-κB), allowing NF-κB translocation to the nucleus and the transcription of inflammatory genes like iNOS and COX-2. Activation of MC4R may block NF-κB activation since cAMP or PKA can prevent IκBα degradation reducing pro-inflammatory gene expression. On the other hand, MC4R might also interact with mitogen-activated protein-kinases (MAPK) and could induce ERK activation or inhibit JNK and p38 MAPKs leading to the activation or inhibition of transcription factors (TF) like AP1 or CREB. LPS plus interferon-γ (IFN-γ) can induce apoptosis by modulating the expression of proteins of the Bcl-2 family. LPS + IFN-γ increase the expression of proapoptotic protein Bax while reducing the expression of antiapoptotic Bcl-2 leading to caspase-3 activation and subsequent cell death by apoptosis. α-MSH prevents apoptosis by increasing Bcl-2 and decreasing Bax expression. Consequently, activation of MC4R decreases caspase-3 activation.

modulates the expression of Bcl-2 [25]. Therefore, it is possible that CREB activation could mediate the anti-inflammatory and antiapoptotic actions of α-MSH.

MC4R activation in vitro induced an increase in the phosphorylation of mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) in the hypothalamus [15], and in CHO-K1 cells [111]. On the other hand, the treatment with α-MSH attenuated the increase in the phosphorylation of p38 MAPK, resulting in a decrease of TNF-α production in leukocytes [61]. The MC4R agonist, NDP-α-MSH diminished the activation of the stress-activated kinase, JNK3 in a model of brain ischemia [37]. MC4R agonists activated ERKs through a mechanism that involved phosphatidylinositol 3-kinase in a hypothalamic cell line [120]. It is possible that cAMP inhibits NF-κB activation through inhibition of the mitogen-activated protein kinase kinase-c-Jun N-terminal kinase (MAPK/JNK) pathway, as overexpression of MAPK reversed the inhibitory effects of cAMP on NF-κB activation [50]. Thus, MAPKs might participate, at least in part, in the anti-inflammatory effect of α-MSH. The intracellular signaling pathways involved in MC4R activation are shown schematically in Fig. 2.

Although there are several small molecule and non-peptide inhibitors of cell signaling known to block NF-κB activation, there are very few normal physiologic peptide hormones reported to block NF-κB activation. It was recently shown that IL-4, IL-10, and growth hormone can block NF-κB activation [21,45,123]. It is known that α-MSH increases production of the anti-inflammatory cytokine IL-10 by modulating the inflammatory cascade [13,124]. On the other hand, low plasma concentrations of such cytokine appear to be associated with early worsening of neurological symptoms in patients with acute ischemic stroke [119]. Some anti-inflammatory effects of α-MSH are mediated via IL-10 production, because IL-10 knockout mice are resistant to α-MSH treatment [101]. Moreover, Vulliemoz et al. [121], showed that SHU9119, a mixed MC3/4 receptor antagonist, can decrease the IL-10 response, establishing a physiological role for endogenous α-MSH in modulating the release of an anti-inflammatory cytokine.

8. Conclusions

There is new evidence for a broader role of α-MSH and MC4R in inflammatory processes. The finding of potent anti-inflammatory effects of α-MSH in different models of brain inflammation encourages the development of synthetic analogs. Due to their long-lasting activity α-MSH-like peptides may turn out to be very useful compounds for their use in the treatment of neuroinflammatory conditions. In addition, α-MSH has no evident toxicity since it has been demonstrated to be safe when given in large and continuous doses to animals and humans. However, α-MSH is very unstable in vivo and its therapeutic use would require daily administration. Since anti-inflammatory and neuroprotective effects of α-MSH might involve MC4R activation, selective MC4R agonists appear to be suited for the treatment of immune-mediated brain inflammatory diseases, without having corticosteroids side effects. Although, α-MSH and MC4R agonists have been shown to possess promising in vitro as well in vivo anti-inflammatory effects, additional studies are necessary to define the role of MC4R and the mechanisms by which α-MSH-like peptides modulate responses to neuroinflammation. The data collected here should help in the design of a new generation of α-MSH-like peptides that could activate MC4R.

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