Astrocytes: new targets of melanocortin 4 receptor actions

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Abstract

Astrocytes exert a wide variety of functions with paramount importance in brain physiology. After injury or infection, astrocytes become reactive and they respond by producing a variety of inflammatory mediators that help maintain brain homeostasis. Loss of astrocyte functions as well as their excessive activation can contribute to disease processes; thus, it is important to modulate reactive astrocyte response. Melanocortins are peptides with well-recognized anti-inflammatory and neuroprotective activity. Although melanocortin efficacy was shown in systemic models of inflammatory disease, mechanisms involved in their effects have not yet been fully elucidated. Central anti-inflammatory effects of melanocortins and their mechanisms are even less well known, and, in particular, the effects of melanocortins in glial cells are poorly understood. Of the five known melanocortin receptors (MCRs), only subtype 4 is present in astrocytes. MC4R has been shown to mediate melanocortin effects on energy homeostasis, reproduction, inflammation, and neuroprotection and, recently, to modulate astrocyte functions. In this review, we will describe MC4R involvement in anti-inflammatory, anorexigenic, and anti-apoptotic effects of melanocortins in the brain. We will highlight MC4R action in astrocytes and discuss their possible mechanisms of action. Melanocortin effects on astrocytes provide a new means of treating inflammation, obesity, and neurodegeneration, making them attractive targets for therapeutic interventions in the CNS.

Key Words
- astrocytes
- MC4R
- inflammation
- neuroprotection
- energy homeostasis

Introduction

Melanocortins are conserved regulatory peptides with anti-inflammatory, anti-pyretic, and neuroprotective effects (Catania et al. 2004, Catania 2008). Astrocytes are the most abundant cell type in the CNS, regarded for a long time merely as support cells for neurons. In recent decades, a growing body of evidence has demonstrated that astrocytes are fundamental pieces in the maintenance of brain homeostasis. Although melanocortin action in astrocytes was reported as early as 1984, only recently were melanocortin receptors (MCRs) identified in these cells. The effects of melanocortins in astrocytes are only beginning to be understood. In this review, we will discuss astrocytes as targets of melanocortin action in the brain.

Astrocytes

Astrocytes are organized in a non-overlapping manner in the brain and have been classified as protoplasmic or
fibrous depending on their morphology and localization. Protoplasmic astrocytes are found in gray matter and have several fine branches with uniform distribution whereas fibrous astrocytes are present in white matter and have few but longer processes. Nevertheless, the diversity of astrogial cells seems to be wider. The human cerebral cortex has several subtypes of astrocytes not found in rodents, and human astrocytes are larger, more diverse, and more complex than rodent astrocytes (Oberheim et al. 2009). Morphological studies showed that astrocytes have processes closely contacting blood vessels known as vascular end-feet that enable astrocytes to interact directly with endothelial cells and to contribute to maintain and regulate the blood–brain barrier (Abbott et al. 2010). Astrocytes also contact neuronal synapses with their neuronal end-feet (Grosche et al. 1999, 2002, Ventura & Harris 1999) and thereby they can modulate neuronal activity. Astrocytes also connect with each other through gap junctions that allow calcium signaling and metabolic coupling between them.

Astrocytes are positioned between blood vessels and neurons allowing them to rapidly respond to changes in the extracellular space. They uptake K⁺ that is accumulated in the synaptic space as a consequence of neuronal activity through K⁺-coupling between them. They uptake K⁺ that is accumulated in the synaptic space as a consequence of neuronal activity through K⁺-channels present in astrocytes (Kofuji & Newman 2004). Also, astrocyte membranes have Na⁺/H⁺ exchangers and bicarbonate transporters to regulate proton shunting (Obara et al. 2008). One of the most important functions of astrocytes is the removal of glutamate from synaptic space through glutamate transporters present in their plasma membrane (Anderson & Swanson 2000), this being the main mechanism by which astrocytes modulate synaptic transmission (Kang et al. 1998). Excessive glutamate release induces excitotoxicity, which may cause neuron death. Activated astrocytes have increased protein levels of glutamate transporters (Krum et al. 2002) that enable them to eliminate excess glutamate in the extracellular space. Within the cytoplasm, glutamate is converted to glutamine by glutamine synthetase. Glutamine is then released by astrocytes and can be taken up by neurons and used to renew glutamate stores.

The regulated release of molecules stored in vesicles in glial cells is known as gliotransmission. Astrocytes can release several gliotransmitters such as glutamate, γ-aminobutyric acid, d-serine, neuropeptides, and ATP in a calcium-dependent manner (Parpura & Zorec 2010). Astrocytes release d-serine at glutamatergic synapses where it then acts as a co-agonist for N-methyl-D-aspartic acid receptors (Henneberger et al. 2010). ATP secreted by astrocytes into the extracellular space contributes to the regulation of postsynaptic efficiency at glutamatergic synapses (Gordon et al. 2005). Also, astrocytes can produce neurotrophic factors such as brain-derived neurotropic factor (BDNF) and nerve growth factor in response to damage, disease, or cytokines (Schwartz & Nishiyama 1994, Rudge et al. 1995, Marz et al. 1999, Albrecht et al. 2002).

**Astrocytes and the inflammatory response**

Inflammation is a physiological response to pathogens, injury, or damage, but when it is exacerbated or becomes chronic, it can contribute to the onset of neurodegenerative disorders. There are several mediators of this response such as cytokines, chemokines, nitric oxide (NO), and prostaglandins (PGs). In the brain, astrocytes and microglia are immune effector cells that recognize pathogenic antigens, become reactive or activated, and elicit an inflammatory response. They recruit immune cells contributing to the induction of pathogen-specific immune adaptive responses (Iwasaki & Medzhitov 2004).

Activation of glial cells leads to signal transduction pathways that activate nuclear factor-κB (NF-κB), a transcription factor that regulates the production of inflammatory mediators. Bacterial lipopolysaccharide (LPS) has been used extensively to produce systemic and brain inflammation. LPS activates its receptor toll-like receptor 4 (TLR4) resulting in NF-κB activation. After systemic LPS administration, tumor necrosis factor-α (TNF-α), IL1β, and IL6 are increased in the brain (Laye et al. 1994). Although all brain cells can synthesize NO, astrocytes and microglia can produce high amounts of NO in response to LPS or pro-inflammatory cytokines by increasing expression of inducible NO synthase (iNOS) (NOS2)) whereas the other two NOS isoforms are constitutively active and produce discrete amounts of this molecule. Similar to NOS, cyclo-oxygenase 1 (COX1 (PTGS1)) is constitutively expressed in the brain whereas COX2 (PTGS2) expression can be induced by pro-inflammatory stimuli such as LPS leading to PG synthesis (Caruso et al. 2004). Although neurons can also produce PGs, astrocytes synthesize higher levels of PGs than neurons (Luo et al. 1998).

Reactive astrogliosis involves cellular hypertrophy, proliferation, and increased production of intermediate filaments such as vimentin and glial fibrillary acidic protein (GFAP). In severe injuries, astrogliosis leads to the formation of the glial scar by proliferating astrocytes, which can prevent axon growth in the damaged area, but also limits damage to a specific area and thus protects the...
surrounding tissue (Buffo et al. 2010). Reactive astrocytes can exacerbate damage by releasing pro-inflammatory cytokines, NO, and reactive oxygen species. In sites distant from the damage or when damage is minor, astrocytes grow in size and increase their production of antioxidants such as glutathione that protect cells from oxidative stress (Wilson 1997) and growth factors that increase neuron survival (Schwartz & Nishiyama 1994). This mild reactive astrogliosis is associated with better recovery from damage. In fact, deletion of reactive astrocytes in a model of spinal cord injury causes failure in blood-brain barrier repair, leukocyte infiltration, severe demyelination, and death of oligodendrocytes and neurons (Faulkner et al. 2004). Gfap knockout mice develop more severe experimental autoimmune encephalomyelitis (EAE) than wild-type mice, which argues in favor of a protective role for astrocytes in this model (Liedtke et al. 1998). On the other hand, over-expression of TNF-α in astrocytes results in neurodegeneration, gliosis, and the development of chronic encephalopathy (Staldler et al. 1998). iNOS expression in astrocytes was observed in Alzheimer’s disease (AD; Luth et al. 2002), multiple sclerosis (MS; Bo et al. 1994), EAE (Tran et al. 1997), and ischemia (Zhu et al. 2003). When NF-κB expression was impaired only in astrocytes, animals were normal and showed a better recovery from spinal cord injury (Brambilla et al. 2005). Inflammatory substances released by astrocytes can have harmful effects and even cause death of brain cells contributing to neurodegeneration. However, other factors also released from these glial cells can promote cell survival; thus, astrocyte activation cannot be regarded simply as beneficial or detrimental. The net result of their activation depends on several factors such as brain environment, type of injury, and time of exposition to injury. Attenuation of pro-inflammatory mediator release without abolishing the release of beneficial factors constitutes a balanced strategy for the treatment of neuroinflammatory diseases.

### Astrocytes and energy homeostasis

The brain has a high energy requirement and its energy supply is also regulated by astrocytes. Glucose enters the brain via endothelial cells and astrocyte end-feet processes. Astrocytes may convert glucose into lactate by performing glycolysis; it may alternatively be used to synthesize glycogen. In fact, glycogen in astrocytes is considered as storage for lactate rather than glucose (Dringen et al. 1993). Lactate is transported to extracellular space via specific transporters where it can be taken up by neurons. Then, neurons convert lactate into pyruvate that can be used to obtain energy, a mechanism known as the astrocyte–neuron lactate shuttle (Pellerin et al. 2007). The astrocyte network actually mediates diffusion of glucose and lactate from vasculature to neurons, especially in sites with high demand of neuron energy (Rouach et al. 2008, Gandhi et al. 2009). Lactate was also shown to be neuroprotective after cerebral ischemia in mice (Berthet et al. 2009).

Fatty acids can also be processed as energy substrates. High levels of fatty acids are found in obesity, metabolic syndrome, and high-fat diet (HFD), and it is known that fatty acids can cross the blood–brain barrier (Dhopeshwarkar & Mead 1973). Obesity-induced inflammation is a local inflammatory response, maintained in a chronic state, induced by nutrients involving metabolic cells interfering with normal metabolism and disrupting insulin and leptin signaling (Gregor & Hotamisligil 2011). Increased expression of pro-inflammatory cytokines in hypothalamus is observed in HFD-treated rats compared with lean controls (De Souza et al. 2005). Also, TNF-α knockout improved insulin sensitivity and lowered circulating free fatty acids in mice fed a HFD (Uysal et al. 1997). As cytokines are targets of NF-κB, this factor is thought to be critically involved in obesity. Indeed, overnutrition induces hypothalamic activation of NF-κB in HFD animals (Zhang et al. 2008).

Given that obesity influences brain functions, a potential role for hypothalamic astrocytes in these effects is postulated (Yi & Tschop 2012, Garcia-Caceres et al. 2013). In fact, GFAP-immunoreactive astrocytes are increased in obese Zucker rats (Tomassoni et al. 2013) and exposure of mice to HFD induces an increase in Gfap mRNA levels as well as in astrocyte numbers and/or processes (Horvath et al. 2010). Interestingly, recent work shows that saturated but not unsaturated fatty acids induce TNF-α and IL6 release from astrocytes via TLR4 activation (Gupta et al. 2012), implicating these cells in obesity-induced inflammation. Moreover, HFD is considered a risk factor for the onset of AD, and palmitic acid-treated astrocytes were shown to induce amyloid processing leading to toxic fragment accumulation (Patil et al. 2006). Hypothalamic astrocytes were reported to express adiponectin receptor 1 that recognizes adiponectin, an adipose tissue-secreted hormone involved in the control of energy homeostasis (Guillod-Maximin et al. 2009). Leptin modulates synaptic inputs in hypothalamus and induces anorexigic signaling in neurons whereas in obesity increased leptin levels are found together with leptin resistance (Schwartz 2006). Astrocytes express leptin...
receptor in hypothalamus, and obesity induced by HFD increases leptin receptor expression in hypothalamic astrocytes (Hsuchou et al. 2009). Also, both overnutrition and chronic leptin treatment of rats increased GFAP and vimentin expression in hypothalamus (Garcia-Caceres et al. 2011). A recent report shows that neonatal over-nutrition increased body weight and leptin, affecting glial cells as GFAP, glucose, and glutamate transporter expression were increased in hypothalamus, further suggesting that physiological changes in metabolic state can modulate astrocyte functions (Fuente-Martín et al. 2012). Moreover, astrocyte leptin receptor knockout mice showed less severe obesity than wild-type mice (Jayaram et al. 2012). While much remains to be understood about the astrocyte role in energy homeostasis, it seems clear that these cells are key players in the CNS response to obesity.

Melanocortin system

The melanocortin system consists of melanocortins, five MCRs, and two endogenous antagonists. Melanocortins include α-, β-, and γ-melanocyte stimulating hormones (MSH) and adrenocorticotropic (ACTH), and are generated by proteolytic cleavage of the precursor peptide pro-opiomelanocortin (POMC) by pro-hormone convertases (PCs). Both PC1 and PC2 are needed to produce α-MSH (Benjannet et al. 1991), after which this peptide suffers additional modifications to become mature α-MSH (Wilkinson 2006). The main source of α-MSH is the pars intermedia of the pituitary gland (Usategui et al. 1976), although it is also synthesized in several other peripheral tissues. α-MSH is synthesized in the arcuate nucleus of the hypothalamus (O’Donohue & Dorsa 1982) and in the nucleus of the solitary tract in the brain stem (Bronstein et al. 1992); from there, POMC neurons project throughout the brain (Bagnol et al. 1999). This system has also two endogenous antagonists: Agouti and Agouti-related peptide (AGRP). Agouti is produced in the skin (Blanchard et al. 1995) where it regulates pigmentation. AGRP is present in the brain only in neurons of the arcuate nucleus (Dinulescu & Cone 2000) where it acts as a competitive antagonist of MC3R and MC4R.

Melanocortin receptors

Five MCRs have been described to date, products of five different genes. All MCRs belong to the family A of G protein-coupled receptors with seven transmembrane domains. MCRs activate adenylate cyclase and induce cAMP production. MC2R is activated only by ACTH.

γ-MSH is a selective MC3R agonist (Roselli-Rehfuss et al. 1993) whereas α-MSH, β-MSH, and ACTH are agonists of all other MCRs (Schioth et al. 1996). MC1R was the first MCR to be cloned from melanocytes (Chhajlani & Wikberg 1992, Mountjoy et al. 1992), and it is expressed in the skin where its activation by α-MSH induces melanogenesis. MC1R is also found in immune cells where it mediates the anti-inflammatory action of α-MSH in leukocytes (Catania 2007). Adrenal gland MC2R activation by ACTH results in production of steroids (Mountjoy et al. 1992). MC2R is also present in rodent adipocytes (Boston & Cone 1996), in human keratinocytes (Sломинski et al. 1996), and in bone cells (Isales et al. 2010). MC3R is widely distributed within the brain (Roselli-Rehfuss et al. 1993) and is also present in several peripheral organs (Gantz et al. 1993a). MC3R knockout mice are obese and hyperphagic (Chen et al. 2000a) and MC3R is thought to function as an autoreceptor in POMC neurons (Cowley et al. 2001). It also has protective effects in rat heart ischemia (Guarini et al. 2002) and is involved in the anti-inflammatory effects of melanocortins in macrophages (Getting et al. 2006). MC4R is expressed predominantly in the brain (Mountjoy et al. 1994), although it was also detected in adipose tissue (Chhajlani 1996), in human skin melanocytes (Spencer & Schallreuter 2009), and in rat heart, lung, kidney, and testis (Mountjoy et al. 2003). MC5R is widely found in peripheral tissue (Gantz et al. 1994, Labbe et al. 1994) and has also been detected in some areas of the CNS (Griffon et al. 1994). Data from Mc5r knockout mice show that this receptor regulates secretion of both lachrymal and sebaceous glands (Chen et al. 1997b).

MC4R

MC4R is an intronless gene that encodes a protein of 332 amino acids with four potential glycosylation sites and two potential palmitoylation sites. It has high levels of homology with the other MCRs. This receptor is also very similar between species. Mouse and rat MC4R have 99% identity whereas rat and human MC4R have 93% identity and their conformation is very similar (Fig. 1). The signaling pathway for MC4R involves G protein-mediated activation of adenylate cyclase and increased cAMP production (Gantz et al. 1993b). It was shown that α-MSH activates CREB (CREB1) in neurons of the hypothalamic paraventricular nucleus (PVN; Sarkar et al. 2002), the solitary nucleus (Sutton et al. 2005), and in hypothalamic cultured neurons (Caruso et al. 2010). We recently reported that MC4R activation in astrocytes also involves
cAMP-protein kinase A (PKA)-CREB activation (Caruso et al. 2012). In addition, MC4R stimulation activates the MAPK ERK-1/2 in vivo (Daniels et al. 2003, Sutton et al. 2005) and in vitro (Daniels et al. 2003, Vongs et al. 2004, Chai et al. 2006, Patten et al. 2007), an effect that may involve phosphoinositol-3 kinase activation (Vongs et al. 2004). An increase in intracellular Ca$^{2+}$ levels was also detected after MC4R stimulation (Mountjoy et al. 2001). There is also interaction between signaling pathways as MC4R activation enhances insulin-stimulated mTOR signaling (Chai et al. 2010) and potentiates leptin signaling (Zhang et al. 2009). Apart from G protein, other proteins may interact with MC4R. Melanocortin 2 receptor accessory protein (MRAP) and MRAP-2 reduce cAMP accumulation induced by melanocortins and are therefore negative regulators of all MCRs except MC2R (Chan et al. 1999). It was also reported that mahogonoid protein reduces MC4R coupling to cAMP (Perez-Oliva et al. 2009). The proteoglycan syndecan-3 enhances AGRP antagonism at MC4R (Reizes et al. 2003). However, further study is needed to fully understand the role of accessory proteins in MC4R functions.

All melanocortins activate MC4R with the exception of $\alpha$-MSH. Some synthetic molecules also act as selective MC4R compounds. (Nle$^4$, d-Phe$^7$)-$\alpha$-MSH (NDP-MSH) is the most potent linear analog of $\alpha$-MSH (Sawyer et al. 1980) with high affinity for all MCRs. Melanotan II (MTII) is a non-selective agonist of all MCRs except MC2R. Ro27-3225 is a selective agonist for MC4R (Benoit et al. 2000) and was shown to protect against hemorrhagic shock (Giuliani et al. 2007). THIQ is a MC4R agonist that reduced food intake in rats (Muceniece et al. 2007). d-Tyr MTII is another selective MC4R agonist recently proven to stimulate MC4R in hippocampal neurons (Shen et al. 2013). Also, another highly selective MC4R agonist, BIM-22493, proved to be effective centrally (Kievit et al. 2013). Of all the antagonists, SHU9119 is a widely used potent antagonist of both MC3R and MC4R (Schioth et al. 1999). HS014 was the first selective MC4R antagonist designed as it has about 20-fold higher affinity for MC4R over MC3R (Schioth et al. 1999). HS024 antagonizes MC4R (Kask et al. 1998) with 100 times more affinity for MC4R than for MC3R, although it antagonizes all MCRs except MC2R.
MC4R-mediated actions

Melanocortins exert a variety of brain effects that have been reviewed in detail elsewhere (Bertolini et al. 2009). Several and diverse melanocortin effects in the brain involve MC4R activation (for review, see Tao (2010)). Some examples of these effects are shown in Table 1. Melanocortins through MC4R activation influence energy homeostasis, acting within the hypothalamus and promoting weight loss. The anorexigenic effect of α-MSH is mediated by the MC4R (Marsh et al. 1999). In fact, targeted disruption of the Mc4r gene causes obesity–diabetes syndrome (Huszar et al. 1998), and mutations in the Mc4r early-onset obesity (Yeo et al. 1997), and sexual function (Schioth & Watanobe 2002, Van der Ploeg et al. 2002) and to augment pain sensitivity (Starowicz et al. 2002, Bertorelli et al. 2005). Antagonists of these receptor are also being evaluated as a treatment for cachexia (DeBoer 2010).

Table 1 Central MC4R-mediated effects

<table>
<thead>
<tr>
<th>Effect on</th>
<th>MC4R agonist or antagonist action</th>
<th>References</th>
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<tbody>
<tr>
<td>Energy homeostasis</td>
<td>α- and β-MSH decrease food intake</td>
<td>Abbott et al. (2000)</td>
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<tr>
<td>Sexual function</td>
<td>MTII agonist reduces food intake and increases metabolic rate</td>
<td>Chen et al. (2000)</td>
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<tr>
<td>Reproduction</td>
<td>THIQ agonist increases erectile activity and enhances copulatory behavior</td>
<td>Rossi et al. (1998)</td>
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<td>Cachexia</td>
<td>MTII agonist increases luteinizing hormone and prolactin secretion in female fasted rats</td>
<td>Van der Ploeg et al. (2002)</td>
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<tr>
<td>Pain</td>
<td>MTII increases and antagonist SHU9119 decreases sensitivity to pain</td>
<td>Wessells et al. (2003)</td>
</tr>
<tr>
<td>Neuroprotection</td>
<td>MTII agonist increases luteinizing hormone and prolactin surge in female fasted rats</td>
<td>Schioth et al. (2001)</td>
</tr>
<tr>
<td>Memory</td>
<td>MC4R function restores pubertal onset, fertility, and lactation in mice</td>
<td>Watanobe et al. (2001)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>MC4R function attenuates cardiac cachexia in heart failure and in mice-bearing tumor Small-melanocortin inhibitors attenuate cachexia</td>
<td>Stanley et al. (1999)</td>
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<tr>
<td>Neuroprotection</td>
<td>MC4R antagonist blocks NDP-MSH protective effect on cerebral ischemia</td>
<td>Israel et al. (2012)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>MC4R antagonist prevents the increase in neurite outgrowth induced by α-MSH in Neuro2A cells</td>
<td>Scarlett et al. (2010)</td>
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<tr>
<td>Memory</td>
<td>HS014 blocks α-MSH-induced recovery from memory impairment produced by IL1β in rats</td>
<td>Joppa et al. (2007)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>HS024 blocks α-MSH-induced reduction of iNOS and COX2 expression induced by LPS in rat hypothalamus and by LPS + IFN-γ in astrocytes</td>
<td>DeBoer (2010)</td>
</tr>
<tr>
<td>Fever</td>
<td>Selective MC4R agonist MRLOB-001 suppresses LPS-induced fever HS014 blocks α-MSH anti-pyretic effect</td>
<td>Starowicz et al. (2002)</td>
</tr>
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MC4R and inflammation

Melanocortins have a well-documented role as potent anti-inflammatory agents in several models of inflammation in peripheral organs (reviewed in Catania et al. (2004)). The anti-inflammatory action of α-MSH reduces secretion of mediators such as cytokines, NO, and PGs and impairs leukocyte activation and infiltration into damaged tissues. Different MCRs may be responsible for the anti-inflammatory properties of melanocortins depending on the tissue or cell type involved. More recent research has provided knowledge on the central action of melanocortins in inflammation. Systemically administered α-MSH reduces cytokine expression in cerebral ischemia (Huang & Tatro 2002) and in brain inflammation induced by LPS (Rajora et al. 1997). α-MSH was shown to inhibit PGE₂ release induced by LPS or IL1β from hippocampal fragments (Weidenfeld et al. 1995) but not from hypothalamic fragments (Mirtella et al. 1995). However, we reported that melanocortins inhibit the production of NO and PGs induced by IL1β in rat hypothalamus (Cragnolini et al. 2006). α-, β-, and γ-MSH were found to exert an anti-inflammatory action in a...
model of neuroinflammation in mice by reducing LPS-induced NO production (Muceniece et al. 2004). As MC3R and MC4R expression in the CNS is high, they are more likely responsible for central melanocortin actions. MC4R involvement in anti-inflammatory actions of melanocortins in the brain has been suggested (Lasaga et al. 2008). Central administration of α-MSH markedly reduces induction of hypothalamic iNOS and Cox2 gene expression in rats injected with LPS, an effect prevented by central administration of the selective MC4R antagonist HS024 (Caruso et al. 2004), indicating for the first time a role for MC4R in inflammation. We also showed that α-MSH attenuates TNF-α expression induced by LPS and interferon-γ (IFN-γ) in hypothalamic cultured neurons that express MC4R (Caruso et al. 2010). Although a role for MC3R in these effects cannot be completely ruled out, evidence suggests that MC4R is involved in the anti-inflammatory effects of melanocortins in the brain.

Effects of melanocortins in astrocytes have been known since 1984 when α-MSH was shown to induce cAMP accumulation in astroglial cultures (Evans et al. 1984). Proliferative effects of α-MSH were reported in 7-day-old cultured astrocytes, an effect no longer observed at later times (Zohar & Salomon 1992), suggesting that melanocortins might have a developmental role in these cells. α-MSH was reported to inhibit TNF-α release induced by LPS in human astrocytoma cells (Wong et al. 1997), although it had no effect on basal or IL1β-induced PGE2 levels in astrocytes (Katsuura et al. 1989). More recently, cloning of the MCRs led to the identification of the subtypes present in astrocytes. Selkirk et al. (2007) demonstrated that only Mc4r mRNA is expressed in rat astrocytes. Considering that they are central cells in the initiation and maintenance of the inflammatory response and that we detected MC4R expression at both mRNA and protein levels in rat astrocytes (Caruso et al. 2007), we hypothesized that astrocytes might be targets of MC4R action. In fact, α-MSH attenuates LPS+IFN-γ-induced inflammatory response in astrocytes as α-MSH treatment decreased iNOS and COX2 expression and consequently NO and PGE2 release and HS024 also prevented these effects (Caruso et al. 2007).

Fever is a host defense response to inflammation mediated mainly by cytokines (IL1β, TNF-α, and IL6) and PGE2. Recently, the RANKL/RANK system was described as another important mediator of fever caused by LPS or cytokines in mouse brain (Hanada et al. 2009). As levels of α-MSH increase in the brain during fever (Bell & Lipton 1987) and circulating levels of α-MSH increase in response to endotoxin administration in humans (Catania et al. 1995), a physiological role for melanocortins in fever has been suggested. Melanocortins are also considered endogenous antipyretics whose effect on fever has been known for some time (Tatro 2000). Central administration of α-MSH reduces fever caused by LPS (Huang et al. 1997), IL1β (Daynes et al. 1987), and TNF-α (Martin et al. 1991). Although the mechanisms involved in the antipyretic action of α-MSH remain unknown, the decrease in pro-inflammatory cytokines and PGs production in the brain can contribute to fever reduction. Indeed, i.p. administration of α-MSH was shown to inhibit fever by activating central MCRs (Huang et al. 1998), and the antipyretic effect of centrally administered α-MSH was also blocked by HS014, a selective MC4R antagonist, thereby highlighting MC4R involvement in α-MSH effect on LPS-induced fever (Sinha et al. 2004). Astrocytes also participate in fever as they produce the inflammatory mediators that cause it, but, surprisingly, this issue has been scantily investigated. One recent report showed that astrocytes are involved in fever induced by RANKL and cytokines. Hanada et al. (2009) showed that inactivation of the RANK receptor in neuronal progenitor cells as well as inactivation of this receptor only in astrocytes abolished fever in response to RANKL, IL1β, and TNF-α (Hanada et al. 2009), indicating that astrocytes are major contributors to inflammation-induced fever. Thus, MC4R activation in astrocytes could help reduce fever by inhibiting release of mediators such as cytokines and PGs.

MC4R and energy homeostasis

The melanocortin system in the arcuate nucleus (ARC) of hypothalamus plays a central role in energy homeostasis. POMC neurons in the ARC release α-MSH in response to peripheral signals such as leptin (Cowley et al. 2001) or insulin (Benoit et al. 2002), after which α-MSH induces an anorexigenic effect by activating MC4R in target neurons. As a result, food intake decreases and metabolic rate increases, promoting weight loss. Leptin and insulin are considered to act as adiposity signals as their blood levels increase in proportion to body fat mass and access the brain where these hormones promote negative energy balance. In addition, in the ARC, AGRP neurons induce the opposite effect when activated, as they have orexigenic effects and also inhibit POMC neurons. AGRP neurons are inhibited by leptin and insulin. Neuronal targets of POMC and AGRP neurons involve the PVN and lateral hypothalamic area (LHA). Neurons in the PVN produce peptides that decrease food intake and increase metabolic rate such as oxytocin, corticotrophin-releasing hormone.
and thyrotropin-releasing hormone (Schwartz 2006). On the contrary, neurons of the LHA stimulate food intake and promote weight gain by releasing orexins and melanin-concentrated hormone. MC4R is expressed in both PVN and LHA neurons where it has a prominent role in energy homeostasis. Mc4r knockout mice are hypophagic and obese with a decreased energy expenditure (Huszar et al. 1997). Recently, treatment with a selective MC4R agonist (BIM-22493) was shown to induce transient decreases in food intake and weight loss over 8 weeks of treatment in diet-induced obese rhesus macaques, which also showed decreased adiposity and improved glucose tolerance (Kievit et al. 1998). Variation of nucleotide sequence in one allele of human MC4R can cause obesity by disrupting MC4R signaling (Ho & MacKenzie 1999, Hinney et al. 2006). In obesity, increased circulating leptin levels are not correlated with increased MC4R activation as there is also leptin resistance. Astrocytes as well as POMC neurons express adipokine receptors, including leptin receptors, and, in response to HFD, they showed increased expression of leptin receptors (Hsuchou et al. 2009). In obesity, reactive astrocytes with enlarged ensheathment impede POMC neuron ability to sense leptin in blood, which was proposed to contribute to leptin resistance (Yi & Tschop 2012). However, a study by Horvath et al. (2010) found that gliosis in HFD might not be the cause of leptin resistance as POMC neuron firing in these mice was as expected in response to a strong leptin input. As melanocortins reduce astrocyte activation (Forslin Aronsson et al. 2006, 2007) and production of inflammatory mediators (Caruso et al. 2007), they could be beneficial for reducing obesity-induced inflammation. Moreover, as we proved that melanocortins induce BDNF expression in astrocytes (Caruso et al. 2012) and BDNF is a mediator of MC4R effects on energy balance (Xu et al. 2003), BDNF released by astrocytes may possibly contribute to anorexigenic effects of MC4R.

Cell response to decreased substrate availability or excess of nutrients is triggered by AMP-activated protein kinase (AMPK), thereby acting as a sensor and regulator of cellular energy levels. In hypothalamus, activation of AMPK regulates the entire body’s energy balance by reducing energy expenditure and enhancing food intake (Minokoshi et al. 2004). Indeed, MC4R stimulation by α-MSH induces inhibition of AMPK in GT1-7 hypothalamic cells (Damm et al. 2012). In another study, Escartin et al. (2007) showed that in ciliary neurotropic factor-activated astrocytes in the striatum, AMPK is activated and these cells were more resistant to glycolysis inhibition and less affected by palmitate toxicity. Also, AMPK was detected in spinal astrocytes and was activated by ADP treatment resulting in ATP production in these cells (Cui et al. 2011). Therefore, we may speculate that MC4R activation in astrocytes can modulate AMPK, which might in turn influence hypothalamic response to energy levels.

**MC4R and neuroprotection**

Melanocortins participate in the development and regeneration of the CNS. α-MSH can act as a neurotrophic factor during development as well as in the adult brain (Strand et al. 1991). A recent study showed that NDP-MSH induces neurogenesis in the hippocampus of gerbils after global ischemia and that this effect is mediated by MC4R (Giuliani et al. 2011). This treatment also improved the animals’ memory and learning. α-MSH through MC4R was also shown to reverse amnesia (Gonzalez et al. 2009), as well as memory reconsolidation impairment (Machado et al. 2010), induced by IL1β administration in the hippocampus of male rats. Melanocortins also exert neuroregenerative actions such as re-growth stimulation of injured axons in rat adult spinal cord (Joosten et al. 1999). α-MSH-induced neurite-like outgrowth was blocked with a specific MC4R antagonist (Adan et al. 1996) and by a selective MC4R antagonist in dorsal root ganglia neurons (Tanabe et al. 2007). Also, topical application of a selective MC4R agonist was found to be neuroprotective in spinal cord injury (Sharma et al. 2006). Therefore, MC4R is involved in the neuroregenerative effects of melanocortins.

Melanocortin treatment has proven to be neuroprotective through MC4R activation in brain injury. In a model of focal cerebral ischemia in gerbils, delayed treatment with α-MSH (Giuliani et al. 2007) or treatment with NDP-MSH but not with the MC3R agonist γ-MSH (Giuliani et al. 2006) reduced neuron death. Also, NDP-MSH reduced neuron death after kainate-induced excitotoxicity (Forslin Aronsson et al. 2007). NDP-MSH was shown to protect a hypothalamic cell line, which expresses MC4R, from serum deprivation-induced apoptosis (Chai et al. 2006). In a rat model of traumatic brain injury, NDP-MSH increased the number of viable neurons in the cortex and the hippocampus (Bitto et al. 2012). This protection correlated with decreased TNF-α and NO production, and decreased expression of pro-apoptotic Bax and caspase-3 activation, and also with increased serum levels of IL10 and Bcl2 expression induced by NDP-MSH. All these effects were blocked by HS024 (Bitto et al. 2012).
In cerebral ischemia, neuroprotection by NDP-MSH also involves activation of MC4R and Bcl2 upregulation (Giuliani et al. 2006). Moreover, melanocortins reduce hippocampal damage and improve learning and memory as long as 50 days after ischemia (Giuliani et al. 2009).

Apoptotic characteristics such as DNA fragmentation were shown to occur in astrocytes adjacent to cerebral ischemia (Li et al. 1995, Chen et al. 1997a) as well as increments in Bax and active caspase-3 levels (Benjelloun et al. 2001), cytokines (Ehrlich et al. 1999, Saas et al. 1999), and NO (Kim et al. 1995, Albrecht et al. 1995, Chen et al. 1999) as well as by excitotoxicity, systemic administration of α-MSH decreased neuron death and astrocyte activation by decreasing the number of GFAP-positive cells (Forslin Aronsson et al. 2006, 2007). However, nothing was known about melanocortin action on astrocyte death. We demonstrated that MC4R activation by α-MSH protects astrocytes from apoptosis induced by LPS + IFN-γ (Caruso et al. 2007). Melanocortins prevent astrocyte death by decreasing caspase-3 activity and the expression of Bax induced by LPS + IFN-γ and by increasing the expression of Bcl2. As melanocortins increase astrocyte survival, this can contribute to their neuroprotective effects.

Astrocytes are able to produce neurotropic factors in response to damage, disease, or cytokines that can promote neuron survival (Schwartz & Nishiyama 1994, Rudge et al. 1995, Albrecht et al. 2002). ACTH was observed to downregulate ciliary neurotropic factor mRNA levels without modifying other neurotrophins in cultured astrocytes (Kokubo et al. 2002). By contrast, an analog of ACTH increased Bdnf mRNA levels in rat glial cell cultures (Shadrina et al. 2001) and after cerebral ischemia (Dmitrieva et al. 2010). Concordantly, we showed that MC4R activation induces expression of BDNF in cultured rat astrocytes (Caruso et al. 2012), suggesting that neuroprotection by MC4R can involve neurotropic factor release.

**Mechanisms of MC4R-mediated effects**

The broad effects exerted by melanocortins can be explained by the fact that α-MSH inhibits NF-κB, a transcription factor that regulates the inflammatory response, by activating transcription of inflammatory mediators (Li & Verma 2002). α-MSH was shown to reduce the activation of NF-κB in vitro (Manna & Aggarwal 1998) and in vivo in the brain (Ichiyama et al. 1999a). However, the situation seems to be different for astrocytes. α-MSH in A172 human glioma cells reduced (Ichiyama et al. 1999b), whereas in H4 glioma cells did not modify (Sarkar et al. 2003), NF-κB activation. We also showed that NF-κB activation was not modified in rat astrocytes (Caruso et al. 2012). Thus, in addition to NF-κB inhibition, an alternative mechanism of action may exist for melanocortins in astrocytes. It was shown that NF-κB activity can also be inhibited by the anti-inflammatory cytokine IL10 from human peripheral mononuclear cells (Yamaoka-Tojo et al. 2006), indicating that melanocortins can also be physiological modulators of IL10. Indeed, we recently reported that NDP-MSH via MC4R activation did not modify IL10 release from astrocytes whereas it did increase IL10 release from microglial cultured cells (Carmiglia et al. 2013). Apart from NF-κB and IL10 modulation, melanocortins activate CREB transcription factor, which is involved in neuron proliferation and survival, learning and memory, as well as in neuroprotection (Lonze & Ginty 2002). CREB is activated by α-MSH in hypothalamic neurons, and although α-MSH decreased TNF-α expression, it did not affect NF-κB activation in these cells (Caruso et al. 2010). In astrocytes, α-MSH increases cAMP intracellular levels and also induces CREB activation (Caruso et al. 2012). Congruently, we blocked BDNF expression induced by MC4R activation in astrocytes with adenylate cyclase and PKA inhibitors (Caruso et al. 2012), confirming that the cAMP-PKA-CREB pathway is activated in astrocytes by MC4R stimulation.

Mechanisms of neuroprotection by melanocortins involve modulation of MAPK activation and expression of proteins from the Bcl2 family. In ischemia models,
melanocortins were reported to reduce MAPK activation (p38, JNK, and ERK1/2) and to increase Bcl2 expression promoting survival of brain cells (Giuliani et al. 2006). In a rat model of traumatic brain injury, NDP-MSH also decreased JNK and ERK1/2 activation as it increased serum levels of IL10 and Bcl2 expression (Bitto et al. 2012). However, ERK activation was induced by melanocortins in rat hypothalamus (Daniels et al. 2003) and in solitary nucleus of the rat (Sutton et al. 2005). ERK1/2 was also activated in GT1-7 hypothalamic cells in response to α-MSH (Damm et al. 2012). Our very recent data also indicate that α-MSH abolishes the reduction in ERK2 phosphorylation induced by IL1β in the hippocampus (Gonzalez et al. 2013) and that ERK1/2 is activated by NDP-MSH in astrocytes (Caruso et al. 2013). Moreover, ERK1/2 activation is known to have protective effects, which is also true for MC4R-mediated ERK1/2 activation as ERK1/2 inhibitor decreases the anti-apoptotic effect of MC4R activation in GT1-1 cells (Chai et al. 2006). Hence, ERK activation and modulation of Bcl2 expression by melanocortins seem to be important mechanisms underlying neuron and astrocyte survival by melanocortins.

As BDNF has proved to be protective in neurodegenerative diseases such as AD, MS, and PD (Nagahara & Tuszynski 2011) and its expression is increased in response to melanocortins in the hypothalamus and also in astrocytes, it is another possible mediator of melanocortin actions. BDNF could have protective effects on neurons and on astrocytes themselves, the latter being an issue that has not been thoroughly investigated. BDNF stimulates S100β expression in mouse astrocytes (Djalali et al. 2005), and it increases intracellular calcium levels of rat astrocytes (Climent et al. 2000), but much study is still needed to fully understand the effects of BDNF in astroglial cells.

Transforming growth factor-β (TGF-β) is another cytokine that modulates inflammatory responses and CNS homeostasis (Aigner & Bogdahn 2008). This cytokine inhibits the LPS-induced expression of TNF-α in astrocytes and microglia (Benveniste et al. 1995, Lodge & Sriram 1996).

**Figure 2**

MC4R activation in astrocytes. Activation of MC4R by α-MSH induces production of cAMP, which leads to CREB activation. This pathway is most likely involved in the anti-inflammatory and anti-apoptotic effects of melanocortins in astrocytes. Although NF-κB is involved in the anti-inflammatory effects of melanocortins, this remains a controversial fact for astrocytes. Instead, MC4R activation induces release of the anti-inflammatory agents PPARγ and TGF-β probably through the cAMP-CREB pathway. BDNF released after MC4R activation occurs through cAMP-PKA-CREB in astrocytes and this neurotrophin through TrkB receptor can have direct effects on neurons, promoting their survival. MC4R activation inhibits apoptosis as it increases BCL2 protein levels and reduces Bax protein levels, thus promoting cell survival against apoptotic stimuli in astrocytes as well as in neurons.
TGF-\(\beta\) can also have neuroprotective effects that are mediated by glial cells (Qian et al. 2008). We recently showed that NDP-MSH increases TGF-\(\beta\) release from astrocytes, and, thus, it is also a possible mediator of melanocortin actions.

A growing body of evidence has implicated peroxisome proliferator-activated receptors (PPARs) in the regulation of inflammatory processes in the CNS (Bright et al. 2008). In glial cells, PPARs (\(\alpha\), \(\beta\), and \(\gamma\)) modulate the production of pro-inflammatory mediators (Lovett-Racke et al. 2004, Aleshin et al. 2009). PPAR\(\gamma\) agonists were found to inhibit the release of pro-inflammatory cytokines by microglial cells and astrocytes (Storer et al. 2005). Anti-inflammatory action of PPAR\(\beta\) agonists has also been demonstrated in these cells (Polak et al. 2005) and in a model of focal cerebral ischemia (Arsenijevic et al. 2006). Our recent findings show for the first time that MC4R activation modulates PPAR expression in glial cells. NDP-MSH increases PPAR\(\gamma\) (PPARG) protein levels whereas it decreases PPAR\(\beta\) (PPARD) protein levels in astrocytes (Carniglia et al. 2013), an effect that has also been described for LPS (Jana & Pahan 2012). In addition, anti-inflammatory effects of PPARs led to the use of their agonists in \textit{in vitro} and \textit{in vivo} models of neurodegenerative diseases with successful results especially in AD (Heneka & Landreth 2007). Thus, PPARs are also strong candidates to mediate MC4R action in the brain.

All together these data suggest that MC4R activation in astrocytes modulates BDNF and PPAR expression, activates CREB, and induces TGF-\(\beta\) release. The role of these factors in MC4R-mediated effects remains to be determined and their actions need to be explored further to prove their therapeutic value.

**Conclusions**

MC4R mediates anti-inflammatory, anorexigenic, and neuroprotective effects of melanocortins within the brain. As astrocytes play a major role in inflammation, the control of their response and the induction of anti-inflammatory and neuroprotective factors by MC4R activation (Fig. 2) may restore astrocyte functionality and thereby lead to amelioration of inflammatory disorders and neurodegenerative diseases. Also, recent evidence shows that astrocytes might be involved in the regulation of energy homeostasis (Fig. 3). However, in view of the variety of effects produced by MC4R activation, development of more selective and potent agonists and
antagonists is needed. Knowledge about the MC4R mechanism of action is of great importance in order for MC4R agonists to become effective therapeutically. Although some progress has been made in this direction, more studies are needed to validate glial MC4R as a potential new therapeutic target.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding
This work was supported by grants of the University of Buenos Aires, CONICET and ANPyCT.

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MC4R action on astrocytes

The melanocortin-4 receptor (MC4R) plays a crucial role in the regulation of energy homeostasis and the control of adiposity. This receptor is primarily expressed in the hypothalamic arcuate nucleus, where it modulates the expression of pro-opiomelanocortin (POMC) and agouti-related protein (AgRP) neurons. These neurons are involved in the regulation of food intake and energy expenditure. The MC4R is located on the plasma membrane of neurons and astrocytes, and its activation leads to the release of neuropeptides such as adrenocorticotropic hormone (ACTH) and growth hormone-releasing hormone (GHRH), which play a role in the regulation of energy metabolism and body weight.

In addition to its role in the hypothalamus, the MC4R is also expressed in various other tissues, including peripheral tissues and immune cells. This receptor plays a role in the regulation of inflammation and immune responses, as well as in the control of adipose tissue. The MC4R in astrocytes is involved in the regulation of cytokine expression and the control of immune cell function.

The MC4R is activated by the melanocortin-3 (MC3R) and -4 receptors (MC4R), which are coupled to G-proteins and stimulate adenyl cyclase. This leads to the production of cAMP, which in turn activates protein kinase A (PKA) and other signaling pathways. The MC4R also interacts with other receptors, such as the bradykinin B2 receptor and the peroxynitrite (NOx) receptor, which modulate the expression of cytokines and other signaling molecules.

In conclusion, the MC4R is a critical regulator of energy homeostasis and immune responses. Its role in the regulation of cytokine expression and the control of immune cell function highlights the importance of understanding its signaling pathways and the mechanisms by which it modulates these responses.


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Published by Bioscientifica Ltd.


Received in final form 27 June 2013
Accepted 23 July 2013
Accepted Preprint published online 23 July 2013