Astrocytes: new targets of melanocortin 4 receptor actions

Carla Caruso, Lila Carniglia, Daniela Durand, Teresa N Scimonelli¹ and Mercedes Lasaga

School of Medicine, Biomedical Research Institute (UBA-CONICET), University of Buenos Aires, Paraguay 2155 piso 10, 1121ABG Buenos Aires, Argentina

¹IFEC (CONICET) Department of Pharmacology, School of Chemistry, National University of Córdoba, Córdoba, Argentina

Correspondence should be addressed to M Lasaga
Email
mlasaga@fmed.uba.ar

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

Journal of Molecular Endocrinology
(2013) 51, R33–R50

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 astroglial 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^+ -channels present in astrocytes (Kofuji & Newman 2004). Also, astrocyte membranes have Na^+/H^+ exchangers and bicarbonate transporters to regulate proton shuttling (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 neurotrophic 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- κ B (NF- κ B), 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- κ B activation. After systemic LPS administration, tumor necrosis factor- α (TNF- α), IL1 β , 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 (Stalder *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- κ B 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- κ B, this factor is thought to be critically involved in obesity. Indeed, overnutrition induces hypothalamic activation of NF- κ B 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 anorexic 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 (Hsueh *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 overnutrition 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-Martin *et al.* 2012). Moreover, astrocyte leptin receptor knockout mice showed less severe obesity than wild-type mice (Jayaram *et al.* 2013). 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 adrenocorticotropin (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-Rehffuss *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 (Slominski *et al.* 1996), and in bone cells (Isales *et al.* 2010). MC3R is widely distributed within the brain (Roselli-Rehffuss *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

Figure 1

Differences between human, mouse, and rat MC4R proteins. Sequence alignment of human, mouse, and rat MC4R protein. Amino acids that differ from the human MC4R sequence are highlighted in gray. Mouse and rat MC4R share 93% of the amino acid sequence with human MC4R and, being

very similar, share the same structure. The N-terminal portion is extracellular whereas the C-terminal portion is intracellular. TM, transmembrane domain; IL, intracellular loop; EL, extracellular loop.

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 for MC2R (Chan *et al.* 2009). Also, it was reported that mahoganoid 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 γ -MSH. Some synthetic molecules also act as selective MC4R compounds. (Nle⁴, D-Phe⁷)- α -MSH (NDP-MSH) is the most potent linear analog of α -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 *Mcr4* gene causes obesity–diabetes syndrome (Huszar *et al.* 1997), and mutations in the *Mcr4* gene are associated with severe early-onset obesity (Yeo *et al.* 1998). MC4R activation was shown to regulate food intake by inducing the release of BDNF in the hypothalamus (Xu *et al.* 2003) and a great amount of current research on MC4R is conducted on this field. MC4R activation was also shown to increase sexual and reproductive 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 this receptor are also being evaluated as a treatment for cachexia (DeBoer 2010).

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 IL1 β 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 IL1 β in rat hypothalamus (Cragnolini *et al.* 2006). α -, β -, and γ -MSH were found to exert an anti-inflammatory action in a

Table 1 Central MC4R-mediated effects

Effect on	MC4R agonist or antagonist action	References
Energy homeostasis	α - and β -MSH decrease food intake MTII agonist reduces food intake and increases metabolic rate AGRP antagonist increases food intake	Abbott <i>et al.</i> (2000) Chen <i>et al.</i> (2000b) Rossi <i>et al.</i> (1998)
Sexual function	THIQ agonist increases erectile activity and enhances copulatory behavior MTII induces and SHU9119 completely blocks penile erection	Van der Ploeg <i>et al.</i> (2002) Wessells <i>et al.</i> (2003)
Reproduction	MTII agonist increases luteinizing hormone and prolactin secretion in female fasted rats AGRP reduces luteinizing hormone and prolactin surge in female fasted rats AGRP induces luteinizing hormone and follicular stimulating hormone release in male rats	Schioth <i>et al.</i> (2001) Watanobe <i>et al.</i> (2001) Stanley <i>et al.</i> (1999)
Cachexia	MC4R function restores pubertal onset, fertility, and lactation in mice AGRP attenuates cardiac cachexia in heart failure and in mice-bearing tumor Small-melanocortin inhibitors attenuate cachexia	Israel <i>et al.</i> (2012) Scarlett <i>et al.</i> (2010) Joppa <i>et al.</i> (2007) DeBoer (2010)
Pain	MTII increases and antagonist SHU9119 decreases sensitivity to pain AGRP reduces mechanical allodynia in a model of chronic pain in rats	Starowicz <i>et al.</i> (2002) Bertorelli <i>et al.</i> (2005)
Neuroprotection	HS024-selective MC4R antagonist blocks NDP-MSH protective effect on cerebral ischemia MC4R antagonist prevents the increase in neurite outgrowth induced by α -MSH in Neuro2A cells	Giuliani <i>et al.</i> (2006) Giuliani <i>et al.</i> (2009) Adan <i>et al.</i> (1996)
Memory	HS014 blocks α -MSH-induced recovery from memory impairment produced by IL1 β in rats NDP-MSH improves memory and learning of gerbils	Gonzalez <i>et al.</i> (2009) Machado <i>et al.</i> (2010) Giuliani <i>et al.</i> (2011)
Inflammation	HS024 blocks α -MSH-induced reduction of iNOS and COX2 expression induced by LPS in rat hypothalamus and by LPS + IFN- γ in astrocytes	Caruso <i>et al.</i> (2004) Caruso <i>et al.</i> (2007)
Fever	Selective MC4R agonist MRLOB-001 suppresses LPS-induced fever HS014 blocks α -MSH anti-pyretic effect	Sinha <i>et al.</i> (2003) Sinha <i>et al.</i> (2004)

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 IL1 β -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 (IL1 β , 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), IL1 β (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, IL1 β , 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 hyperphagic 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.* 2013). Mutations in *MC4R* gene are associated with severe early-onset obesity (Yeo *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 (Hsueh *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 & Tschöp 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 neurotrophic 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 neurotropic 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 IL1 β 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.* 2003). Inflammatory stimuli such as LPS (Suk *et al.* 2001), cytokines (Ehrlich *et al.* 1999, Saas *et al.* 1999), and NO (Kim *et al.* 2001, Durand *et al.* 2010) can induce apoptosis of astrocytes. Astrocyte apoptosis can have beneficial as well as detrimental effects on neurons. In neuron-astrocyte co-cultures, the presence of astrocytes diminishes neuron death induced by oxidative stress (Blanc *et al.* 1998), and blocking astrocyte gap junctions induces neuron death in response to glutamate (Ozog *et al.* 2002). Indeed, cultured spinal cord astrocytes exposed to peroxynitrites for 24 h promote activation of caspase-3 and apoptosis of motor neurons that grow on top of them (Cassina *et al.* 2002). By contrast, astrogliosis is observed in AD, Huntington's disease, and Parkinson's disease. Moreover, in MS, Huntington's disease, ischemia, and brain injury, astrocytes undergo apoptosis (Takuma *et al.* 2004, Maragakis & Rothstein 2006). Therefore, reduction of the essential functions performed by astrocytes as well as their activation can directly contribute to neurodegeneration. In models of neuron death induced by cerebral ischemia or 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- κ B, 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- κ B *in vitro* (Manna & Aggarwal 1998) and *in vivo* in the brain (Ichihama *et al.* 1999a). However, the situation seems to be different for astrocytes. α -MSH in A172 human glioma cells reduced (Ichihama *et al.* 1999b), whereas in H4 glioma cells did not modify (Sarkar *et al.* 2003), NF- κ B activation. We also showed that NF- κ B activation was not modified in rat astrocytes (Caruso *et al.* 2012). Thus, in addition to NF- κ B inhibition, an alternative mechanism of action may exist for melanocortins in astrocytes. It was shown that NF- κ B activity can also be inhibited by the anti-inflammatory cytokine IL10 in monocytes (Wang *et al.* 1995). Indeed, SHU9119, a MC3R/4R antagonist, reduces *per se* IL10 serum release induced by LPS (Vulliamoz *et al.* 2006), and IL10 is released 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 (Carniglia *et al.* 2013). Apart from NF- κ B 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- κ B 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 IL1 β 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 S100 β 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- κ B 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 PPAR γ 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.

Figure 3

Astrocytes and MC4R in obesity. In response to a HFD diet, the circulation of saturated fatty acids and cytokines is increased. Astrocytes can be activated by both saturated fatty acids and cytokines, and, thus, they respond by increasing GFAP and vimentin expression and by activating NF- κ B, which in turn induces cytokine expression. Both cytokines (inflammation) and

saturated fatty acids (HFD) promote diet-induced obesity. Stimulation of MC4R by α -MSH reduces astrocyte activation and pro-inflammatory mediators, so it could also be possible that these cells help to induce a decrease in energy balance and thus this may decrease diet-induced obesity susceptibility.

TGF- β can also have neuroprotective effects that are mediated by glial cells (Qian *et al.* 2008). We recently showed that NDP-MSH increases TGF- β 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 (α , β , and γ) modulate the production of pro-inflammatory mediators (Lovett-Racke *et al.* 2004, Aleshin *et al.* 2009). PPAR γ 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 β 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 γ (PPARG) protein levels whereas it decreases PPAR β (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 *in vitro* and *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- β 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.

References

- Abbott CR, Rossi M, Kim M, AlAhmed SH, Taylor GM, Ghatei MA, Smith DM & Bloom SR 2000 Investigation of the melanocyte stimulating hormones on food intake. Lack of evidence to support a role for the melanocortin-3-receptor. *Brain Research* **869** 203–210. (doi:10.1016/S0006-8993(00)02386-6)
- Abbott NJ, Patabendige AA, Dolman DE, Yusof SR & Begley DJ 2010 Structure and function of the blood–brain barrier. *Neurobiology of Disease* **37** 13–25. (doi:10.1016/j.nbd.2009.07.030)
- Adan RA, van der Kraan M, Doornbos RP, Bar PR, Burbach JP & Gispen WH 1996 Melanocortin receptors mediate α -MSH-induced stimulation of neurite outgrowth in neuro 2A cells. *Brain Research. Molecular Brain Research* **36** 37–44. (doi:10.1016/0169-328X(95)00236-L)
- Aigner L & Bogdahn U 2008 TGF- β in neural stem cells and in tumors of the central nervous system. *Cell and Tissue Research* **331** 225–241. (doi:10.1007/s00441-007-0466-7)
- Albrecht PJ, Dahl JP, Stoltzfus OK, Levenson R & Levison SW 2002 Ciliary neurotrophic factor activates spinal cord astrocytes, stimulating their production and release of fibroblast growth factor-2, to increase motor neuron survival. *Experimental Neurology* **173** 46–62. (doi:10.1006/exnr.2001.7834)
- Aleshin S, Grabeklis S, Hanck T, Sergeeva M & Reiser G 2009 Peroxisome proliferator-activated receptor (PPAR)-gamma positively controls and PPARalpha negatively controls cyclooxygenase-2 expression in rat brain astrocytes through a convergence on PPARbeta/delta via mutual control of PPAR expression levels. *Molecular Pharmacology* **76** 414–424. (doi:10.1124/mol.109.056010)
- Anderson CM & Swanson RA 2000 Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32** 1–14. (doi:10.1002/1098-1136(200010)32:1<1::AID-GLIA10>3.0.CO;2-W)
- Arsenijevic D, de Bilbao F, Plamondon J, Paradis E, Vallet P, Richard D, Langhans W & Giannakopoulos P 2006 Increased infarct size and lack of hyperphagic response after focal cerebral ischemia in peroxisome proliferator-activated receptor β -deficient mice. *Journal of Cerebral Blood Flow and Metabolism* **26** 433–445. (doi:10.1038/sj.jcbfm.9600200)
- Bagnol D, Lu XY, Kaelin CB, Day HE, Ollmann M, Gantz I, Akil H, Barsh GS & Watson SJ 1999 Anatomy of an endogenous antagonist: relationship between Agouti-related protein and proopiomelanocortin in brain. *Journal of Neuroscience* **19** RC26.
- Bell RC & Lipton JM 1987 Pulsatile release of antipyretic neuropeptide α -MSH from septum of rabbit during fever. *American Journal of Physiology* **252** R1152–R1157.
- Benjannet S, Rondeau N, Day R, Chretien M & Seidah NG 1991 PC1 and PC2 are proprotein convertases capable of cleaving proopiomelanocortin at distinct pairs of basic residues. *PNAS* **88** 3564–3568. (doi:10.1073/pnas.88.9.3564)
- Benjelloun N, Joly LM, Palmier B, Plotkine M & Charriaut-Marlangue C 2003 Apoptotic mitochondrial pathway in neurones and astrocytes after neonatal hypoxia–ischaemia in the rat brain. *Neuropathology and Applied Neurobiology* **29** 350–360. (doi:10.1046/j.1365-2990.2003.00467.x)
- Benoit SC, Schwartz MW, Lachey JL, Hagan MM, Rushing PA, Blake KA, Yagaloff KA, Kurylko G, Franco L, Danhoo W *et al.* 2000 A novel selective melanocortin-4 receptor agonist reduces food intake in rats and mice without producing aversive consequences. *Journal of Neuroscience* **20** 3442–3448.
- Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ, Seeley RJ & Woods SC 2002 The catabolic action of insulin in the brain is mediated by melanocortins. *Journal of Neuroscience* **22** 9048–9052.
- Benveniste EN, Tang LP & Law RM 1995 Differential regulation of astrocyte TNF- α expression by the cytokines TGF- β , IL-6 and IL-10. *International Journal of Developmental Neuroscience* **13** 341–349. (doi:10.1016/0736-5748(94)00061-7)
- Berthet C, Lei H, Thevenet J, Gruetter R, Magistretti PJ & Hirt L 2009 Neuroprotective role of lactate after cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism* **29** 1780–1789. (doi:10.1038/jcbfm.2009.97)
- Bertolini A, Tacchi R & Vergoni AV 2009 Brain effects of melanocortins. *Pharmacological Research* **59** 13–47. (doi:10.1016/j.phrs.2008.10.005)
- Bertorelli R, Fredduzzi S, Tarozzo G, Campanella M, Grundy R, Beltramo M & Reggiani A 2005 Endogenous and exogenous melanocortin antagonists induce anti-allodynic effects in a model of rat neuropathic pain. *Behavioural Brain Research* **157** 55–62. (doi:10.1016/j.bbr.2004.06.008)
- Bitto A, Polito F, Irrera N, Calo M, Spaccapelo L, Marini HR, Giuliani D, Ottani A, Rinaldi M, Minutoli L *et al.* 2012 Protective effects of melanocortins on short-term changes in a rat model of traumatic brain injury*. *Critical Care Medicine* **40** 945–951. (doi:10.1097/CCM.0b013e318236efde)
- Blanc EM, Bruce-Keller AJ & Mattson MP 1998 Astrocytic gap junctional communication decreases neuronal vulnerability to oxidative stress-induced disruption of Ca^{2+} homeostasis and cell death. *Journal of Neurochemistry* **70** 958–970. (doi:10.1046/j.1471-4159.1998.70030958.x)
- Blanchard SG, Harris CO, Ittoop OR, Nichols JS, Parks DJ, Truesdale AT & Wilkison WO 1995 Agouti antagonism of melanocortin binding and action in the B16F10 murine melanoma cell line. *Biochemistry* **34** 10406–10411. (doi:10.1021/bi00033a012)
- Bo L, Dawson TM, Wesselingh S, Mork S, Choi S, Kong PA, Hanley D & Trapp BD 1994 Induction of nitric oxide synthase in demyelinating regions of multiple sclerosis brains. *Annals of Neurology* **36** 778–786. (doi:10.1002/ana.410360515)
- Boston BA & Cone RD 1996 Characterization of melanocortin receptor subtype expression in murine adipose tissues and in the 3T3-L1 cell line. *Endocrinology* **137** 2043–2050. (doi:10.1210/en.137.5.2043)
- Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A, Karmally S, Green EJ & Bethea JR 2005 Inhibition of astroglial nuclear factor kappaB reduces inflammation and improves functional recovery after spinal cord injury. *Journal of Experimental Medicine* **202** 145–156. (doi:10.1084/jem.20041918)
- Bright JJ, Kanakasabai S, Chearwae W & Chakraborty S 2008 PPAR regulation of inflammatory signaling in CNS diseases. *PPAR Research* **2008** 658520. (doi:10.1155/2008/658520)
- Bronstein DM, Schafer MK, Watson SJ & Akil H 1992 Evidence that β -endorphin is synthesized in cells in the nucleus tractus solitarius: detection of POMC mRNA. *Brain Research* **587** 269–275. (doi:10.1016/0006-8993(92)91007-2)

- Buffo A, Rolando C & Ceruti S 2010 Astrocytes in the damaged brain: molecular and cellular insights into their reactive response and healing potential. *Biochemical Pharmacology* **79** 77–89. (doi:10.1016/j.bcp.2009.09.014)
- Carniglia L, Durand D, Caruso C & Lasaga M 2013 Effect of NDP- α -MSH on PPAR- γ and - β expression and anti-inflammatory cytokine release in rat astrocytes and microglia. *PLoS ONE* **8** e57313. (doi:10.1371/journal.pone.0057313)
- Caruso C, Mohn C, Karara AL, Rettori V, Watanobe H, Schioth HB, Seilicovich A & Lasaga M 2004 α -Melanocyte-stimulating hormone through melanocortin-4 receptor inhibits nitric oxide synthase and cyclooxygenase expression in the hypothalamus of male rats. *Neuroendocrinology* **79** 278–286. (doi:10.1159/000079321)
- Caruso C, Durand D, Schioth HB, Rey R, Seilicovich A & Lasaga M 2007 Activation of melanocortin 4 receptors reduces the inflammatory response and prevents apoptosis induced by lipopolysaccharide and interferon-gamma in astrocytes. *Endocrinology* **148** 4918–4926. (doi:10.1210/en.2007-0366)
- Caruso C, Sanchez M, Durand D, Perez Mde L, Gonzalez PV, Lasaga M & Scimoneilli TN 2010 α -Melanocyte-stimulating hormone modulates lipopolysaccharide plus interferon-gamma-induced tumor necrosis factor- α expression but not tumor necrosis factor- α receptor expression in cultured hypothalamic neurons. *Journal of Neuroimmunology* **227** 52–59. (doi:10.1016/j.jneuroim.2010.06.013)
- Caruso C, Carniglia L, Durand D, Gonzalez PV, Scimoneilli TN & Lasaga M 2012 Melanocortin 4 receptor activation induces brain-derived neurotrophic factor expression in rat astrocytes through cyclic AMP-protein kinase A pathway. *Molecular and Cellular Endocrinology* **348** 47–54. (doi:10.1016/j.mce.2011.07.036)
- Caruso C, Carniglia L, Durand D & Lasaga M 2013 MC4R activation induces BDNF expression through ERK and PI3K in rat astrocytes. *XI European Meeting on Glial Cells in Health and Disease. Glia* **61** (Supplement S1) S186. (doi:10.1002/glia.22530)
- Cassina P, Peluffo H, Pehar M, Martinez-Palma L, Ressler A, Beckman JS, Estevez AG & Barbeito L 2002 Peroxynitrite triggers a phenotypic transformation in spinal cord astrocytes that induces motor neuron apoptosis. *Journal of Neuroscience Research* **67** 21–29. (doi:10.1002/jnr.10107)
- Catania A 2007 The melanocortin system in leukocyte biology. *Journal of Leukocyte Biology* **81** 383–392. (doi:10.1189/jlb.0706426)
- Catania A 2008 Neuroprotective actions of melanocortins: a therapeutic opportunity. *Trends in Neurosciences* **31** 353–360. (doi:10.1016/j.tins.2008.04.002)
- Catania A, Suffredini AF & Lipton JM 1995 Endotoxin causes release of α -melanocyte-stimulating hormone in normal human subjects. *Neuroimmunomodulation* **2** 258–262. (doi:10.1159/000097204)
- Catania A, Gatti S, Colombo G & Lipton JM 2004 Targeting melanocortin receptors as a novel strategy to control inflammation. *Pharmacological Reviews* **56** 1–29. (doi:10.1124/pr.56.1.1)
- Chai B, Li JY, Zhang W, Newman E, Ammori J & Mulholland MW 2006 Melanocortin-4 receptor-mediated inhibition of apoptosis in immortalized hypothalamic neurons via mitogen-activated protein kinase. *Peptides* **27** 2846–2857. (doi:10.1016/j.peptides.2006.05.005)
- Chai B, Li JY, Zhang W, Wu X, Zhang C & Mulholland MW 2010 Melanocortin-4 receptor activation promotes insulin-stimulated mTOR signaling. *Peptides* **31** 1888–1893. (doi:10.1016/j.peptides.2010.06.028)
- Chan LF, Webb TR, Chung TT, Meimaridou E, Cooray SN, Guasti L, Chapple JP, Egertova M, Elphick MR, Cheetham ME *et al.* 2009 MRAP and MRAP2 are bidirectional regulators of the melanocortin receptor family. *PNAS* **106** 6146–6151. (doi:10.1073/pnas.0809918106)
- Chen J, Jin K, Chen M, Pei W, Kawaguchi K, Greenberg DA & Simon RP 1997a Early detection of DNA strand breaks in the brain after transient focal ischemia: implications for the role of DNA damage in apoptosis and neuronal cell death. *Journal of Neurochemistry* **69** 232–245. (doi:10.1046/j.1471-4159.1997.69010232.x)
- Chen W, Kelly MA, Opitz-Araya X, Thomas RE, Low MJ & Cone RD 1997b Exocrine gland dysfunction in MC5-R-deficient mice: evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell* **91** 789–798. (doi:10.1016/S0092-8674(00)80467-5)
- Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan XM, Yu H, Rosenblum CI, Vongs A, Feng Y, Cao L *et al.* 2000a Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nature Genetics* **26** 97–102. (doi:10.1038/79254)
- Chen AS, Metzger JM, Trumbauer ME, Guan XM, Yu H, Frazier EG, Marsh DJ, Forrest MJ, Gopal-Truter S, Fisher J *et al.* 2000b Role of the melanocortin-4 receptor in metabolic rate and food intake in mice. *Transgenic Research* **9** 145–154. (doi:10.1023/A:1008983615045)
- Chhajlani V 1996 Distribution of cDNA for melanocortin receptor subtypes in human tissues. *Biochemistry & Molecular Biology International* **38** 73–80.
- Chhajlani V & Wikberg JE 1992 Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. *FEBS Letters* **309** 417–420. (doi:10.1016/0014-5793(92)80820-7)
- Climent E, Sancho-Tello M, Minana R, Barettoni D & Guerri C 2000 Astrocytes in culture express the full-length Trk-B receptor and respond to brain derived neurotrophic factor by changing intracellular calcium levels: effect of ethanol exposure in rats. *Neuroscience Letters* **288** 53–56. (doi:10.1016/S0304-3940(00)01207-6)
- Cowley MA, Smart JL, Rubinstein M, Cerdan MG, Diano S, Horvath TL, Cone RD & Low MJ 2001 Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411** 480–484. (doi:10.1038/35078085)
- Cragolini AB, Caruso C, Lasaga M & Scimoneilli TN 2006 α -MSH and gamma-MSH modulate early release of hypothalamic PGE₂ and NO induced by IL-1 β differently. *Neuroscience Letters* **409** 168–172. (doi:10.1016/j.neulet.2006.09.034)
- Cui J, Ou S, He WJ, Du L, Zhao YD & Ruan HZ 2011 Prevention of extracellular ADP-induced ATP accumulation of the cultured rat spinal astrocytes via P2Y₁-mediated inhibition of AMPK. *Neuroscience Letters* **503** 244–249. (doi:10.1016/j.neulet.2011.08.045)
- Damm E, Buech TR, Gudermann T & Breit A 2012 Melanocortin-induced PKA activation inhibits AMPK activity via ERK-1/2 and LKB-1 in hypothalamic GT1-7 cells. *Molecular Endocrinology* **26** 643–654. (doi:10.1210/me.2011-1218)
- Daniels D, Patten CS, Roth JD, Yee DK & Fluharty SJ 2003 Melanocortin receptor signaling through mitogen-activated protein kinase *in vitro* and in rat hypothalamus. *Brain Research* **986** 1–11. (doi:10.1016/S0006-8993(03)03162-7)
- Daynes RA, Robertson BA, Cho BH, Burnham DK & Newton R 1987 α -Melanocyte-stimulating hormone exhibits target cell selectivity in its capacity to affect interleukin 1-inducible responses *in vivo* and *in vitro*. *Journal of Immunology* **139** 103–109.
- DeBoer MD 2010 Update on melanocortin interventions for cachexia: progress toward clinical application. *Nutrition* **26** 146–151. (doi:10.1016/j.nut.2009.07.003)
- De Souza CT, Araujo EP, Bordin S, Ashimine R, Zollner RL, Boschero AC, Saad MJ & Velloso LA 2005 Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology* **146** 4192–4199. (doi:10.1210/en.2004-1520)
- Dhopeshwarkar GA & Mead JF 1973 Uptake and transport of fatty acids into the brain and the role of the blood-brain barrier system. *Advances in Lipid Research* **11** 109–142.
- Dinulescu DM & Cone RD 2000 Agouti and agouti-related protein: analogies and contrasts. *Journal of Biological Chemistry* **275** 6695–6698. (doi:10.1074/jbc.275.10.6695)
- Djalali S, Holtje M, Grosse G, Rothe T, Stroth T, Grosse J, Deng DR, Hellweg R, Grantyn R, Hortnagl H *et al.* 2005 Effects of brain-derived neurotrophic factor (BDNF) on glial cells and serotonergic neurones during development. *Journal of Neurochemistry* **92** 616–627. (doi:10.1111/j.1471-4159.2004.02911.x)

- Dmitrieva VG, Povarova OV, Skvortsova VI, Limborska SA, Myasoedov NF & Dergunova LV 2010 Semax and Pro-Gly-Pro activate the transcription of neurotrophins and their receptor genes after cerebral ischemia. *Cellular and Molecular Neurobiology* **30** 71–79. (doi:10.1007/s10571-009-9432-0)
- Dringen R, Gebhardt R & Hamprecht B 1993 Glycogen in astrocytes: possible function as lactate supply for neighboring cells. *Brain Research* **623** 208–214. (doi:10.1016/0006-8993(93)91429-V)
- Durand D, Caruso C, Carniglia L & Lasaga M 2010 Metabotropic glutamate receptor 3 activation prevents nitric oxide-induced death in cultured rat astrocytes. *Journal of Neurochemistry* **112** 420–433. (doi:10.1111/j.1471-4159.2009.06469.x)
- Ehrlich LC, Peterson PK & Hu S 1999 Interleukin (IL)-1 β -mediated apoptosis of human astrocytes. *Neuroreport* **10** 1849–1852. (doi:10.1097/00001756-199906230-00009)
- Escartin C, Pierre K, Colin A, Brouillet E, Delzescaux T, Guillemier M, Dhenain M, Deglon N, Hantraye P, Pellerin L *et al.* 2007 Activation of astrocytes by CNTF induces metabolic plasticity and increases resistance to metabolic insults. *Journal of Neuroscience* **27** 7094–7104. (doi:10.1523/JNEUROSCI.0174-07.2007)
- Evans T, McCarthy KD & Harden TK 1984 Regulation of cyclic AMP accumulation by peptide hormone receptors in immunocytochemically defined astroglial cells. *Journal of Neurochemistry* **43** 131–138. (doi:10.1111/j.1471-4159.1984.tb06688.x)
- Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB & Sofroniew MV 2004 Reactive astrocytes protect tissue and preserve function after spinal cord injury. *Journal of Neuroscience* **24** 2143–2155. (doi:10.1523/JNEUROSCI.3547-03.2004)
- Forslin Aronsson S, Spulber S, Popescu LM, Winblad B, Post C, Oprica M & Schultzberg M 2006 α -Melanocyte-stimulating hormone is neuroprotective in rat global cerebral ischemia. *Neuropeptides* **40** 65–75. (doi:10.1016/j.npep.2005.10.006)
- Forslin Aronsson A, Spulber S, Oprica M, Winblad B, Post C & Schultzberg M 2007 α -MSH rescues neurons from excitotoxic cell death. *Journal of Molecular Neuroscience* **33** 239–251. (doi:10.1007/s12031-007-0019-2)
- Fuente-Martin E, Garcia-Caceres C, Granado M, de Ceballos ML, Sanchez-Garrido MA, Sarman B, Liu ZW, Dietrich MO, Tena-Sempere M, Argente-Arizon P *et al.* 2012 Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes. *Journal of Clinical Investigation* **122** 3900–3913. (doi:10.1172/JCI64102)
- Gandhi GK, Cruz NF, Ball KK & Dienel GA 2009 Astrocytes are poised for lactate trafficking and release from activated brain and for supply of glucose to neurons. *Journal of Neurochemistry* **111** 522–536. (doi:10.1111/j.1471-4159.2009.06333.x)
- Gantz I, Konda Y, Tashiro T, Shimoto Y, Miwa H, Munzert G, Watson SJ, DelValle J & Yamada T 1993a Molecular cloning of a novel melanocortin receptor. *Journal of Biological Chemistry* **268** 8246–8250.
- Gantz I, Miwa H, Konda Y, Shimoto Y, Tashiro T, Watson SJ, DelValle J & Yamada T 1993b Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *Journal of Biological Chemistry* **268** 15174–15179.
- Gantz I, Shimoto Y, Konda Y, Miwa H, Dickinson CJ & Yamada T 1994 Molecular cloning, expression, and characterization of a fifth melanocortin receptor. *Biochemical and Biophysical Research Communications* **200** 1214–1220. (doi:10.1006/bbrc.1994.1580)
- Garcia-Caceres C, Fuente-Martin E, Burgos-Ramos E, Granado M, Frago LM, Barrios V, Horvath T, Argente J & Chowen JA 2011 Differential acute and chronic effects of leptin on hypothalamic astrocyte morphology and synaptic protein levels. *Endocrinology* **152** 1809–1818. (doi:10.1210/en.2010-1252)
- Garcia-Caceres C, Yi CX & Tschop MH 2013 Hypothalamic astrocytes in obesity. *Endocrinology and Metabolism Clinics of North America* **42** 57–66. (doi:10.1016/j.ecl.2012.11.003)
- Getting SJ, Lam CW, Chen AS, Grieco P & Perretti M 2006 Melanocortin 3 receptors control crystal-induced inflammation. *FASEB Journal* **20** 2234–2241. (doi:10.1096/fj.06-6339com)
- Giuliani D, Mioni C, Altavilla D, Leone S, Bazzani C, Minutoli L, Bitto A, Cainazzo MM, Marini H, Zaffe D *et al.* 2006 Both early and delayed treatment with melanocortin 4 receptor-stimulating melanocortins produces neuroprotection in cerebral ischemia. *Endocrinology* **147** 1126–1135. (doi:10.1210/en.2005-0692)
- Giuliani D, Ottani A, Mioni C, Bazzani C, Galantucci M, Minutoli L, Bitto A, Zaffe D, Botticelli AR, Squadrito F *et al.* 2007 Neuroprotection in focal cerebral ischemia owing to delayed treatment with melanocortins. *European Journal of Pharmacology* **570** 57–65. (doi:10.1016/j.ejphar.2007.05.025)
- Giuliani D, Ottani A, Minutoli L, Stefano VD, Galantucci M, Bitto A, Zaffe D, Altavilla D, Botticelli AR, Squadrito F *et al.* 2009 Functional recovery after delayed treatment of ischemic stroke with melanocortins is associated with overexpression of the activity-dependent gene Zif268. *Brain, Behavior, and Immunity* **23** 844–850. (doi:10.1016/j.bbi.2009.03.009)
- Giuliani D, Zaffe D, Ottani A, Spaccapelo L, Galantucci M, Minutoli L, Bitto A, Irrera N, Contri M, Altavilla D *et al.* 2011 Treatment of cerebral ischemia with melanocortins acting at MC4 receptors induces marked neurogenesis and long-lasting functional recovery. *Acta Neuropathologica* **122** 443–453. (doi:10.1007/s00401-011-0873-4)
- Gonzalez PV, Schioth HB, Lasaga M & Scimonelli TN 2009 Memory impairment induced by IL-1 β is reversed by α -MSH through central melanocortin-4 receptors. *Brain, Behavior, and Immunity* **23** 817–822. (doi:10.1016/j.bbi.2009.03.001)
- Gonzalez P, Machado I, Vilcaes A, Roth G, Lasaga M & Scimonelli T 2013 The impairment on fear memory consolidation induced by IL-1 β involves MAPK p38 phosphorylation and reduction of ERK activation. In *International Society of Neurochemistry 24th Biennial Joint Meeting*. Ed Abstract. Mexico: Cancun.
- Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE & Bains JS 2005 Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nature Neuroscience* **8** 1078–1086. (doi:10.1038/nn1498)
- Gregor MF & Hotamisligil GS 2011 Inflammatory mechanisms in obesity. *Annual Review of Immunology* **29** 415–445. (doi:10.1146/annurev-immunol-031210-101322)
- Griffon N, Mignon V, Facchinetti P, Diaz J, Schwartz JC & Sokoloff P 1994 Molecular cloning and characterization of the rat fifth melanocortin receptor. *Biochemical and Biophysical Research Communications* **200** 1007–1014. (doi:10.1006/bbrc.1994.1550)
- Grosche J, Matyash V, Moller T, Verkhratsky A, Reichenbach A & Kettenmann H 1999 Microdomains for neuron–glia interaction: parallel fiber signaling to Bergmann glial cells. *Nature Neuroscience* **2** 139–143. (doi:10.1038/5692)
- Grosche J, Kettenmann H & Reichenbach A 2002 Bergmann glial cells form distinct morphological structures to interact with cerebellar neurons. *Journal of Neuroscience Research* **68** 138–149. (doi:10.1002/jnr.10197)
- Guarini S, Schioth HB, Mioni C, Cainazzo M, Ferrazza G, Giuliani D, Wikberg JE, Bertolini A & Bazzani C 2002 MC(3) receptors are involved in the protective effect of melanocortins in myocardial ischemia/reperfusion-induced arrhythmias. *Naunyn-Schmiedeberg's Archives of Pharmacology* **366** 177–182. (doi:10.1007/s00210-002-0572-8)
- Guilod-Maximin E, Roy AF, Vacher CM, Aubourg A, Bailleux V, Lorsignol A, Penicaud L, Parquet M & Taouis M 2009 Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. *Journal of Endocrinology* **200** 93–105. (doi:10.1677/JOE-08-0348)
- Gupta S, Knight AG, Keller JN & Bruce-Keller AJ 2012 Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. *Journal of Neurochemistry* **120** 1060–1071. (doi:10.1111/j.1471-4159.2012.07660.x)
- Hanada R, Leibbrandt A, Hanada T, Kitaoka S, Furuyashiki T, Fujihara H, Trichereau J, Paolino M, Qadri F, Plehm R *et al.* 2009 Central control of fever and female body temperature by RANKL/RANK. *Nature* **462** 505–509. (doi:10.1038/nature08596)

- Heneka MT & Landreth GE 2007 PPARs in the brain. *Biochimica et Biophysica Acta* **1771** 1031–1045. (doi:10.1016/j.bbali.2007.04.016)
- Henneberger C, Papouin T, Oliet SH & Rusakov DA 2010 Long-term potentiation depends on release of D-serine from astrocytes. *Nature* **463** 232–236. (doi:10.1038/nature08673)
- Hinney A, Bettecken T, Tarnow P, Brumm H, Reichwald K, Lichtner P, Scherag A, Nguyen TT, Schlumberger P, Rief W *et al.* 2006 Prevalence, spectrum, and functional characterization of melanocortin-4 receptor gene mutations in a representative population-based sample and obese adults from Germany. *Journal of Clinical Endocrinology and Metabolism* **91** 1761–1769. (doi:10.1210/jc.2005-2056)
- Ho G & MacKenzie RG 1999 Functional characterization of mutations in melanocortin-4 receptor associated with human obesity. *Journal of Biological Chemistry* **274** 35816–35822. (doi:10.1074/jbc.274.50.35816)
- Horvath TL, Sarman B, Garcia-Caceres C, Enriori PJ, Sotonyi P, Shanabrough M, Borok E, Argente J, Chowen JA, Perez-Tilve D *et al.* 2010 Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. *PNAS* **107** 14875–14880. (doi:10.1073/pnas.1004282107)
- Hsouchou H, He Y, Kastin AJ, Tu H, Markadakis EN, Rogers RC, Fossier PB & Pan W 2009 Obesity induces functional astrocytic leptin receptors in hypothalamus. *Brain* **132** 889–902. (doi:10.1093/brain/awp029)
- Huang Q & Tatro JB 2002 α -Melanocyte stimulating hormone suppresses intracerebral tumor necrosis factor- α and interleukin-1 β gene expression following transient cerebral ischemia in mice. *Neuroscience Letters* **334** 186–190. (doi:10.1016/S0304-3940(02)01088-1)
- Huang QH, Entwistle ML, Alvaro JD, Duman RS, Hrubby VJ & Tatro JB 1997 Antipyretic role of endogenous melanocortins mediated by central melanocortin receptors during endotoxin-induced fever. *Journal of Neuroscience* **17** 3343–3351.
- Huang QH, Hrubby VJ & Tatro JB 1998 Systemic α -MSH suppresses LPS fever via central melanocortin receptors independently of its suppression of corticosterone and IL-6 release. *American Journal of Physiology* **275** R524–R530.
- Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD *et al.* 1997 Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88** 131–141. (doi:10.1016/S0092-8674(00)81865-6)
- Ichiyama T, Sakai T, Catania A, Barsh GS, Furukawa S & Lipton JM 1999a Systemically administered α -melanocyte-stimulating peptides inhibit NF- κ B activation in experimental brain inflammation. *Brain Research* **836** 31–37. (doi:10.1016/S0006-8993(99)01584-X)
- Ichiyama T, Zhao H, Catania A, Furukawa S & Lipton JM 1999b α -Melanocyte-stimulating hormone inhibits NF- κ B activation and IkappaB α degradation in human glioma cells and in experimental brain inflammation. *Experimental Neurology* **157** 359–365. (doi:10.1006/exnr.1999.7064)
- Isales CM, Zaidi M & Blair HC 2010 ACTH is a novel regulator of bone mass. *Annals of the New York Academy of Sciences* **1192** 110–116. (doi:10.1111/j.1749-6632.2009.05231.x)
- Israel DD, Sheffer-Babila S, de Luca C, Jo YH, Liu SM, Xia Q, Spergel DJ, Dun SL, Dun NJ & Chua SC Jr 2012 Effects of leptin and melanocortin signaling interactions on pubertal development and reproduction. *Endocrinology* **153** 2408–2419. (doi:10.1210/en.2011-1822)
- Iwasaki A & Medzhitov R 2004 Toll-like receptor control of the adaptive immune responses. *Nature Immunology* **5** 987–995. (doi:10.1038/nri112)
- Jana M & Pahan K 2012 Gemfibrozil, a lipid lowering drug, inhibits the activation of primary human microglia via peroxisome proliferator-activated receptor β . *Neurochemical Research* **37** 1718–1729. (doi:10.1007/s11064-012-0781-6)
- Jayaram B, Pan W, Wang Y, Hsouchou H, Mace A, Cornelissen-Guillaume GG, Mishra PK, Koza RA & Kastin AJ 2013 Astrocytic leptin-receptor knockout mice show partial rescue of leptin resistance in diet-induced obesity. *Journal of Applied Physiology* **114** 734–741. (doi:10.1152/japplphysiol.01499.2012)
- Joosten EA, Majewska B, Houweling DA, Bar PR & Gispen WH 1999 α -Melanocyte stimulating hormone promotes regrowth of injured axons in the adult rat spinal cord. *Journal of Neurotrauma* **16** 543–553. (doi:10.1089/neu.1999.16.543)
- Joppa MA, Gogas KR, Foster AC & Markison S 2007 Central infusion of the melanocortin receptor antagonist agouti-related peptide (AgRP (83–132)) prevents cachexia-related symptoms induced by radiation and colon-26 tumors in mice. *Peptides* **28** 636–642. (doi:10.1016/j.peptides.2006.11.021)
- Kang J, Jiang L, Goldman SA & Nedergaard M 1998 Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nature Neuroscience* **1** 683–692. (doi:10.1038/3684)
- Kask A, Mutulis F, Muceniece R, Pahkla R, Mutule I, Wikberg JE, Rago L & Schioth HB 1998 Discovery of a novel superpotent and selective melanocortin-4 receptor antagonist (HS024): evaluation *in vitro* and *in vivo*. *Endocrinology* **139** 5006–5014. (doi:10.1210/en.139.12.5006)
- Katsuura G, Gottschall PE, Dahl RR & Arimura A 1989 Interleukin-1 β increases prostaglandin E₂ in rat astrocyte cultures: modulatory effect of neuropeptides. *Endocrinology* **124** 3125–3127. (doi:10.1210/endo-124-6-3125)
- Kievit P, Halem H, Marks DL, Dong JZ, Glavas MM, Sinnayah P, Pranger L, Cowley MA, Grove KL & Culler MD 2013 Chronic treatment with a melanocortin-4 receptor agonist causes weight loss, reduces insulin resistance, and improves cardiovascular function in diet-induced obese rhesus macaques. *Diabetes* **62** 490–497. (doi:10.2337/db12-0598)
- Kim MS, Cheong YP, So HS, Lee KM, Kim TY, Oh J, Chung YT, Son Y, Kim BR & Park R 2001 Protective effects of morphine in peroxynitrite-induced apoptosis of primary rat neonatal astrocytes: potential involvement of G protein and phosphatidylinositol 3-kinase (PI3 kinase). *Biochemical Pharmacology* **61** 779–786. (doi:10.1016/S0006-2952(01)00541-X)
- Kofuji P & Newman EA 2004 Potassium buffering in the central nervous system. *Neuroscience* **129** 1045–1056. (doi:10.1016/j.neuroscience.2004.06.008)
- Kokubo M, Asai K, Yamamoto N, Aoyama M, Morikawa M, Togari H, Wada Y & Kato T 2002 ACTH(1–24) down-regulates expression of ciliary neurotrophic factor mRNA in cultured rat astrocyte. *Pediatric Research* **52** 950–957. (doi:10.1203/00006450-200212000-00022)
- Krum JM, Phillips TM & Rosenstein JM 2002 Changes in astroglial GLT-1 expression after neural transplantation or stab wounds. *Experimental Neurology* **174** 137–149. (doi:10.1006/exnr.2002.7867)
- Labbe O, Desarnaud F, Eggerickx D, Vassart G & Parmentier M 1994 Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. *Biochemistry* **33** 4543–4549. (doi:10.1021/bi00181a015)
- Lasaga M, Debeljuk L, Durand D, Scimonelli TN & Caruso C 2008 Role of α -melanocyte stimulating hormone and melanocortin 4 receptor in brain inflammation. *Peptides* **29** 1825–1835. (doi:10.1016/j.peptides.2008.06.009)
- Laye S, Parnet P, Goujon E & Dantzer R 1994 Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Brain Research. Molecular Brain Research* **27** 157–162. (doi:10.1016/0169-328X(94)90197-X)
- Li Q & Verma IM 2002 NF- κ B regulation in the immune system. *Nature Reviews. Immunology* **2** 725–734. (doi:10.1038/nri910)
- Li Y, Chopp M, Jiang N, Yao F & Zaloga C 1995 Temporal profile of *in situ* DNA fragmentation after transient middle cerebral artery occlusion in the rat. *Journal of Cerebral Blood Flow and Metabolism* **15** 389–397. (doi:10.1038/jcbfm.1995.49)
- Liedtke W, Edelmann W, Chiu FC, Kucherlapati R & Raine CS 1998 Experimental autoimmune encephalomyelitis in mice lacking glial fibrillary acidic protein is characterized by a more severe clinical course and an infiltrative central nervous system lesion. *American Journal of Pathology* **152** 251–259.
- Lodge PA & Sriram S 1996 Regulation of microglial activation by TGF- β , IL-10, and CSF-1. *Journal of Leukocyte Biology* **60** 502–508.

- Lonze BE & Ginty DD 2002 Function and regulation of CREB family transcription factors in the nervous system. *Neuron* **35** 605–623. (doi:10.1016/S0896-6273(02)00828-0)
- Lovett-Racke AE, Hussain RZ, Northrop S, Choy J, Rocchini A, Matthes L, Chavis JA, Diab A, Drew PD & Racke MK 2004 Peroxisome proliferator-activated receptor α agonists as therapy for autoimmune disease. *Journal of Immunology* **172** 5790–5798.
- Luo J, Lang JA & Miller MW 1998 Transforming growth factor β 1 regulates the expression of cyclooxygenase in cultured cortical astrocytes and neurons. *Journal of Neurochemistry* **71** 526–534. (doi:10.1046/j.1471-4159.1998.71020526.x)
- Luth HJ, Munch G & Arendt T 2002 Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. *Brain Research* **953** 135–143. (doi:10.1016/S0006-8993(02)03280-8)
- Machado I, Gonzalez P, Schioth HB, Lasaga M & Scimonelli TN 2010 α -Melanocyte-stimulating hormone (α -MSH) reverses impairment of memory reconsolidation induced by interleukin-1 β (IL-1 β) hippocampal infusions. *Peptides* **31** 2141–2144. (doi:10.1016/j.peptides.2010.07.018)
- Manna SK & Aggarwal BB 1998 α -Melanocyte-stimulating hormone inhibits the nuclear transcription factor NF-kappa B activation induced by various inflammatory agents. *Journal of Immunology* **161** 2873–2880.
- Maragakis NJ & Rothstein JD 2006 Mechanisms of disease: astrocytes in neurodegenerative disease. *Nature Clinical Practice. Neurology* **2** 679–689. (doi:10.1038/ncpneuro0355)
- Marsh DJ, Hollopeter G, Huszar D, Laufer R, Yagaloff KA, Fisher SL, Burn P & Palmiter RD 1999 Response of melanocortin-4 receptor-deficient mice to anorectic and orexigenic peptides. *Nature Genetics* **21** 119–122. (doi:10.1038/5070)
- Martin LW, Catania A, Hiltz ME & Lipton JM 1991 Neuropeptide α -MSH antagonizes IL-6- and TNF-induced fever. *Peptides* **12** 297–299. (doi:10.1016/0196-9781(91)90015-H)
- Marz P, Heese K, Dimitriadis-Schmutz B, Rose-John S & Otten U 1999 Role of interleukin-6 and soluble IL-6 receptor in region-specific induction of astrocytic differentiation and neurotrophin expression. *Glia* **26** 191–200. (doi:10.1002/(SICI)1098-1136(199905)26:3<191::AID-GLIA>3.0.CO;2-))
- Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foulfelle F, Ferre P, Birnbaum MJ *et al.* 2004 AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* **428** 569–574. (doi:10.1038/nature02440)
- Mirtella A, Tringali G, Guerriero G, Ghiara P, Parente L, Preziosi P & Navarra P 1995 Evidence that the interleukin-1 β -induced prostaglandin E₂ release from rat hypothalamus is mediated by type I and type II interleukin-1 receptors. *Journal of Neuroimmunology* **61** 171–177. (doi:10.1016/0165-5728(95)00088-J)
- Mountjoy KG, Robbins LS, Mortrud MT & Cone RD 1992 The cloning of a family of genes that encode the melanocortin receptors. *Science* **257** 1248–1251. (doi:10.1126/science.1325670)
- Mountjoy KG, Mortrud MT, Low MJ, Simerly RB & Cone RD 1994 Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Molecular Endocrinology* **8** 1298–1308. (doi:10.1210/me.8.10.1298)
- Mountjoy KG, Kong PL, Taylor JA, Willard DH & Wilkison WO 2001 Melanocortin receptor-mediated mobilization of intracellular free calcium in HEK293 cells. *Physiological Genomics* **5** 11–19.
- Mountjoy KG, Jenny Wu CS, Dumont LM & Wild JM 2003 Melanocortin-4 receptor messenger ribonucleic acid expression in rat cardiorespiratory, musculoskeletal, and integumentary systems. *Endocrinology* **144** 5488–5496. (doi:10.1210/en.2003-0570)
- Muceniec R, Zvejniec L, Kirjanova O, Liepinsh E, Krigere L, Bauman L, Kalvinsh I, Wikberg JE & Dambrova M 2004 Beta- and gamma-melanocortins inhibit lipopolysaccharide induced nitric oxide production in mice brain. *Brain Research* **995** 7–13. (doi:10.1016/j.brainres.2003.09.039)
- Muceniec R, Zvejniec L, Vilskersts R, Liepinsh E, Bauman L, Kalvinsh I, Wikberg JE & Dambrova M 2007 Functional evaluation of THIQ, a melanocortin 4 receptor agonist, in models of food intake and inflammation. *Basic & Clinical Pharmacology & Toxicology* **101** 416–420. (doi:10.1111/j.1742-7843.2007.00133.x)
- Nagahara AH & Tuszynski MH 2011 Potential therapeutic uses of BDNF in neurological and psychiatric disorders. *Nature Reviews. Drug Discovery* **10** 209–219. (doi:10.1038/nrd3366)
- Obara M, Szeliga M & Albrecht J 2008 Regulation of pH in the mammalian central nervous system under normal and pathological conditions: facts and hypotheses. *Neurochemistry International* **52** 905–919. (doi:10.1016/j.neuint.2007.10.015)
- Oberheim NA, Takano T, Han X, He W, Lin JH, Wang F, Xu Q, Wyatt JD, Pilcher W, Ojemann JG *et al.* 2009 Uniquely hominid features of adult human astrocytes. *Journal of Neuroscience* **29** 3276–3287. (doi:10.1523/JNEUROSCI.4707-08.2009)
- O'Donohue TL & Dorsa DM 1982 The opiomelanotropinergic neuronal and endocrine systems. *Peptides* **3** 353–395. (doi:10.1016/0196-9781(82)90098-5)
- Ozog MA, Siushansian R & Naus CC 2002 Blocked gap junctional coupling increases glutamate-induced neurotoxicity in neuron-astrocyte co-cultures. *Journal of Neuropathology and Experimental Neurology* **61** 132–141.
- Parpura V & Zorec R 2010 Gliotransmission: exocytotic release from astrocytes. *Brain Research Reviews* **63** 83–92. (doi:10.1016/j.brainresrev.2009.11.008)
- Patil S, Sheng L, Masserang A & Chan C 2006 Palmitic acid-treated astrocytes induce BACE1 upregulation and accumulation of C-terminal fragment of APP in primary cortical neurons. *Neuroscience Letters* **406** 55–59. (doi:10.1016/j.neulet.2006.07.015)
- Patten CS, Daniels D, Suzuki A, Fluharty SJ & Yee DK 2007 Structural and signaling requirements of the human melanocortin 4 receptor for MAP kinase activation. *Regulatory Peptides* **142** 111–122. (doi:10.1016/j.regpep.2007.02.005)
- Pellerin L, Bouzier-Sore AK, Aubert A, Serres S, Merle M, Costalat R & Magistretti PJ 2007 Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* **55** 1251–1262. (doi:10.1002/glia.20528)
- Perez-Oliva AB, Olivares C, Jimenez-Cervantes C & Garcia-Borrón JC 2009 Mahogunin ring finger-1 (MGRN1) E3 ubiquitin ligase inhibits signaling from melanocortin receptor by competition with Galphas. *Journal of Biological Chemistry* **284** 31714–31725. (doi:10.1074/jbc.M109.028100)
- Polak PE, Kalinin S, Dello Russo C, Gavrilyuk V, Sharp A, Peters JM, Richardson J, Willson TM, Weinberg G & Feinstein DL 2005 Protective effects of a peroxisome proliferator-activated receptor- β /delta agonist in experimental autoimmune encephalomyelitis. *Journal of Neuroimmunology* **168** 65–75. (doi:10.1016/j.jneuroim.2005.07.006)
- Qian L, Wei SJ, Zhang D, Hu X, Xu Z, Wilson B, El-Benna J, Hong JS & Flood PM 2008 Potent anti-inflammatory and neuroprotective effects of TGF- β 1 are mediated through the inhibition of ERK and p47phox-Ser345 phosphorylation and translocation in microglia. *Journal of Immunology* **181** 660–668.
- Rajora N, Boccoli G, Burns D, Sharma S, Catania AP & Lipton JM 1997 α -MSH modulates local and circulating tumor necrosis factor- α in experimental brain inflammation. *Journal of Neuroscience* **17** 2181–2186.
- Reizes O, Benoit SC, Strader AD, Clegg DJ, Akunuru S & Seeley RJ 2003 Syndecan-3 modulates food intake by interacting with the melanocortin/AgRP pathway. *Annals of the New York Academy of Sciences* **994** 66–73. (doi:10.1111/j.1749-6632.2003.tb03163.x)
- Roselli-Rehffuss L, Mountjoy KG, Robbins LS, Mortrud MT, Low MJ, Tatro JB, Entwistle ML, Simerly RB & Cone RD 1993 Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *PNAS* **90** 8856–8860. (doi:10.1073/pnas.90.19.8856)

- Rossi M, Kim MS, Morgan DG, Small CJ, Edwards CM, Sunter D, Abusnana S, Goldstone AP, Russell SH, Stanley SA *et al.* 1998 A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of α -melanocyte stimulating hormone *in vivo*. *Endocrinology* **139** 4428–4431. (doi:10.1210/en.139.10.4428)
- Rouach N, Koulakoff A, Abudara V, Willecke K & Giaume C 2008 Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* **322** 1551–1555. (doi:10.1126/science.1164022)
- Rudge JS, Pasnikowski EM, Holst P & Lindsay RM 1995 Changes in neurotrophic factor expression and receptor activation following exposure of hippocampal neuron/astrocyte cocultures to kainic acid. *Journal of Neuroscience* **15** 6856–6867.
- Saas P, Boucraut J, Quiquerez AL, Schnuriger V, Perrin G, Desplat-Jego S, Bernard D, Walker PR & Dietrich PY 1999 CD95(Fas/Apo-1) as a receptor governing astrocyte apoptotic or inflammatory responses: a key role in brain inflammation? *Journal of Immunology* **162** 2326–2333.
- Sarkar S, Legradi G & Lechan RM 2002 Intracerebroventricular administration of α -melanocyte stimulating hormone increases phosphorylation of CREB in TRH- and CRH-producing neurons of the hypothalamic paraventricular nucleus. *Brain Research* **945** 50–59. (doi:10.1016/S0006-8993(02)02619-7)
- Sarkar A, Sreenivasan Y & Manna SK 2003 α -Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced biological responses by downregulating CD14 from macrophages. *FEBS Letters* **553** 286–294. (doi:10.1016/S0014-5793(03)01029-9)
- Sawyer TK, Sanfilippo PJ, Hruby VJ, Engel MH, Heward CB, Burnett JB & Hadley ME 1980 4-Norleucine, 7-D-phenylalanine- α -melanocyte-stimulating hormone: a highly potent α -melanotropin with ultralong biological activity. *PNAS* **77** 5754–5758. (doi:10.1073/pnas.77.10.5754)
- Scarlett JM, Bowe DD, Zhu X, Batra AK, Grant WF & Marks DL 2010 Genetic and pharmacologic blockade of central melanocortin signaling attenuates cardiac cachexia in rodent models of heart failure. *Journal of Endocrinology* **206** 121–130. (doi:10.1677/JOE-09-0397)
- Schioth HB & Watanobe H 2002 Melanocortins and reproduction. *Brain Research. Brain Research Reviews* **38** 340–350. (doi:10.1016/S0165-0173(01)00159-X)
- Schioth HB, Chhajlani V, Muceniec R, Klusa V & Wikberg JE 1996 Major pharmacological distinction of the ACTH receptor from other melanocortin receptors. *Life Sciences* **59** 797–801. (doi:10.1016/0024-3205(96)00370-0)
- Schioth HB, Muceniec R, Mutulis F, Bouifrouri AA, Mutule I & Wikberg JE 1999 Further pharmacological characterization of the selective melanocortin 4 receptor antagonist HS014: comparison with SHU9119. *Neuropeptides* **33** 191–196. (doi:10.1054/npep.1999.0760)
- Schioth HB, Kakizaki Y, Kohsaka A, Suda T & Watanobe H 2001 Agouti-related peptide prevents steroid-induced luteinizing hormone and prolactin surges in female rats. *Neuroreport* **12** 687–690. (doi:10.1097/00001756-200103260-00014)
- Schwartz MW 2006 Central nervous system regulation of food intake. *Obesity* **14** (Suppl 1) 1S–8S. (doi:10.1038/oby.2006.275)
- Schwartz JP & Nishiyama N 1994 Neurotrophic factor gene expression in astrocytes during development and following injury. *Brain Research Bulletin* **35** 403–407. (doi:10.1016/0361-9230(94)90151-1)
- Selkirk JV, Nottebaum LM, Lee J, Yang W, Foster AC & Lechner SM 2007 Identification of differential melanocortin 4 receptor agonist profiles at natively expressed receptors in rat cortical astrocytes and recombinantly expressed receptors in human embryonic kidney cells. *Neuropharmacology* **52** 459–466. (doi:10.1016/j.neuropharm.2006.08.015)
- Shadrina MI, Dolotov OV, Grivennikov IA, Slominsky PA, Andreeva LA, Inozemtseva LS, Limborska SA & Myasoedov NF 2001 Rapid induction of neurotrophin mRNAs in rat glial cell cultures by Semax, an adrenocorticotrophic hormone analog. *Neuroscience Letters* **308** 115–118. (doi:10.1016/S0304-3940(01)01994-2)
- Sharma HS, Skottner A, Lundstedt T, Flardh M & Wiklund L 2006 Neuroprotective effects of melanocortins in experimental spinal cord injury. An experimental study in the rat using topical application of compounds with varying affinity to melanocortin receptors. *Journal of Neural Transmission* **113** 463–476. (doi:10.1007/s00702-005-0404-3)
- Shen Y, Fu WY, Cheng EY, Fu AK & Ip NY 2013 Melanocortin-4 receptor regulates hippocampal synaptic plasticity through a protein kinase A-dependent mechanism. *Journal of Neuroscience* **33** 464–472. (doi:10.1523/JNEUROSCI.3282-12.2013)
- Sinha PS, Schioth HB & Tatro JB 2003 Activation of central melanocortin-4 receptor suppresses lipopolysaccharide-induced fever in rats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **284** R1595–R1603. (doi:10.1152/ajpregu.00581.2002)
- Sinha PS, Schioth HB & Tatro JB 2004 Roles of the melanocortin-4 receptor in antipyretic and hyperthermic actions of centrally administered α -MSH. *Brain Research* **1001** 150–158. (doi:10.1016/j.brainres.2003.12.007)
- Slominski A, Ermak G & Mihm M 1996 ACTH receptor, CYP11A1, CYP17 and CYP21A2 genes are expressed in skin. *Journal of Clinical Endocrinology and Metabolism* **81** 2746–2749. (doi:10.1210/jc.81.7.2746)
- Spencer JD & Schallreuter KU 2009 Regulation of pigmentation in human epidermal melanocytes by functional high-affinity β -melanocyte-stimulating hormone/melanocortin-4 receptor signaling. *Endocrinology* **150** 1250–1258. (doi:10.1210/en.2008-1212)
- Stalder AK, Carson MJ, Pagenstecher A, Asensio VC, Kincaid C, Benedict M, Powell HC, Masliah E & Campbell IL 1998 Late-onset chronic inflammatory encephalopathy in immune-competent and severe combined immune-deficient (SCID) mice with astrocyte-targeted expression of tumor necrosis factor. *American Journal of Pathology* **153** 767–783. (doi:10.1016/S0002-9440(10)65620-9)
- Stanley SA, Small CJ, Kim MS, Heath MM, Seal LJ, Russell SH, Gbatei MA & Bloom SR 1999 Agouti related peptide (Agrp) stimulates the hypothalamic pituitary gonadal axis *in vivo* & *in vitro* in male rats. *Endocrinology* **140** 5459–5462. (doi:10.1210/en.140.11.5459)
- Starowicz K, Przewlocki R, Gispén WH & Przewlocka B 2002 Modulation of melanocortin-induced changes in spinal nociception by mu-opioid receptor agonist and antagonist in neuropathic rats. *Neuroreport* **13** 2447–2452. (doi:10.1097/00001756-200212200-00015)
- Storer PD, Xu J, Chavis J & Drew PD 2005 Peroxisome proliferator-activated receptor-gamma agonists inhibit the activation of microglia and astrocytes: implications for multiple sclerosis. *Journal of Neuroimmunology* **161** 113–122. (doi:10.1016/j.jneuroim.2004.12.015)
- Strand FL, Rose KJ, Zuccarelli LA, Kume J, Alves SE, Antonawich FJ & Garrett LY 1991 Neuropeptide hormones as neurotrophic factors. *Physiological Reviews* **71** 1017–1046.
- Suk K, Lee J, Hur J, Kim YS, Lee M, Cha S, Yeou Kim S & Kim H 2001 Activation-induced cell death of rat astrocytes. *Brain Research* **900** 342–347. (doi:10.1016/S0006-8993(01)02326-5)
- Sutton GM, Duos B, Patterson LM & Berthoud HR 2005 Melanocortinergic modulation of cholecystokinin-induced suppression of feeding through extracellular signal-regulated kinase signaling in rat solitary nucleus. *Endocrinology* **146** 3739–3747. (doi:10.1210/en.2005-0562)
- Takuma K, Baba A & Matsuda T 2004 Astrocyte apoptosis: implications for neuroprotection. *Progress in Neurobiology* **72** 111–127. (doi:10.1016/j.pneurobio.2004.02.001)
- Tanabe K, Gamo K, Aoki S, Wada K & Kiyama H 2007 Melanocortin receptor 4 is induced in nerve-injured motor and sensory neurons of mouse. *Journal of Neurochemistry* **101** 1145–1152. (doi:10.1111/j.1471-4159.2006.04432.x)
- Tao YX 2010 The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocrine Reviews* **31** 506–543. (doi:10.1210/er.2009-0037)
- Tatro JB 2000 Endogenous antipyretics. *Clinical Infectious Diseases* **31** (Suppl 5) S190–S201. (doi:10.1086/317519)
- Tomassoni D, Nwankwo IE, Gabrielli MG, Bhatt S, Muhammad AB, Lokhandwala MF, Tayebati SK & Amenta F 2013 Astroglialosis in the brain of obese Zucker rat: a model of metabolic syndrome. *Neuroscience Letters* **543** 136–141. (doi:10.1016/j.neulet.2013.03.025)

- Tran EH, Hardin-Pouzet H, Verge G & Owens T 1997 Astrocytes and microglia express inducible nitric oxide synthase in mice with experimental allergic encephalomyelitis. *Journal of Neuroimmunology* **74** 121–129. (doi:10.1016/S0165-5728(96)00215-9)
- Usategui R, Oliver C, Vaudry H, Lombardi G, Rozenberg I & Mourre AM 1976 Immunoreactive α -MSH and ACTH levels in rat plasma and pituitary. *Endocrinology* **98** 189–196. (doi:10.1210/endo-98-1-189)
- Uysal KT, Wiesbrock SM, Marino MW & Hotamisligil GS 1997 Protection from obesity-induced insulin resistance in mice lacking TNF- α function. *Nature* **389** 610–614. (doi:10.1038/39335)
- Van der Ploeg LH, Martin WJ, Howard AD, Nargund RP, Austin CP, Guan X, Drisko J, Cashen D, Sebhat I, Patchett AA *et al.* 2002 A role for the melanocortin 4 receptor in sexual function. *PNAS* **99** 11381–11386. (doi:10.1073/pnas.172378699)
- Ventura R & Harris KM 1999 Three-dimensional relationships between hippocampal synapses and astrocytes. *Journal of Neuroscience* **19** 6897–6906.
- Vongs A, Lynn NM & Rosenblum CI 2004 Activation of MAP kinase by MC4-R through PI3 kinase. *Regulatory Peptides* **120** 113–118. (doi:10.1016/j.regpep.2004.02.018)
- Vulliamoz NR, Xiao E, Xia-Zhang L, Ferin M & Wardlaw SL 2006 Melanocortin modulation of inflammatory cytokine and neuroendocrine responses to endotoxin in the monkey. *Endocrinology* **147** 1878–1883. (doi:10.1210/en.2005-1430)
- Wang P, Wu P, Siegel MI, Egan RW & Billah MM 1995 Interleukin (IL)-10 inhibits nuclear factor kappa B (NF kappa B) activation in human monocytes, IL-10 and IL-4 suppress cytokine synthesis by different mechanisms. *Journal of Biological Chemistry* **270** 9558–9563. (doi:10.1074/jbc.270.16.9558)
- Watanabe H, Yoneda M, Kakizaki Y, Kohsaka A, Suda T & Schiöth HB 2001 Further evidence for a significant participation of the melanocortin 4 receptor in the preovulatory prolactin surge in the rat. *Brain Research Bulletin* **54** 521–525. (doi:10.1016/S0361-9230(01)00442-7)
- Weidenfeld J, Crumeyrolle-Arias M & Haour F 1995 Effect of bacterial endotoxin and interleukin-1 on prostaglandin biosynthesis by the hippocampus of mouse brain: role of interleukin-1 receptors and glucocorticoids. *Neuroendocrinology* **62** 39–46. (doi:10.1159/000126986)
- Wessells H, Hruby VJ, Hackett J, Han G, Balse-Srinivasan P & Vanderah TW 2003 Ac-Nle-c[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂ induces penile erection via brain and spinal melanocortin receptors. *Neuroscience* **118** 755–762. (doi:10.1016/S0306-4522(02)00866-7)
- Wilkinson CW 2006 Roles of acetylation and other post-translational modifications in melanocortin function and interactions with endorphins. *Peptides* **27** 453–471. (doi:10.1016/j.peptides.2005.05.029)
- Wilson JX 1997 Antioxidant defense of the brain: a role for astrocytes. *Canadian Journal of Physiology and Pharmacology* **75** 1149–1163. (doi:10.1139/y97-146)
- Wong KY, Rajora N, Boccoli G, Catania A & Lipton JM 1997 A potential mechanism of local anti-inflammatory action of α -melanocyte-stimulating hormone within the brain: modulation of tumor necrosis factor- α production by human astrocytic cells. *Neuroimmunomodulation* **4** 37–41.
- Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH & Reichardt LF 2003 Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nature Neuroscience* **6** 736–742. (doi:10.1038/nn1073)
- Yamaoka-Tojo M, Tojo T, Shioi T, Masuda T, Inomata T & Izumi T 2006 Central neurotensin, α -melanocyte-stimulating hormone (α -MSH) is upregulated in patients with congestive heart failure. *Internal Medicine* **45** 429–434. (doi:10.2169/internalmedicine.45.1546)
- Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG & O'Rahilly S 1998 A frameshift mutation in MC4R associated with dominantly inherited human obesity. *Nature Genetics* **20** 111–112. (doi:10.1038/2404)
- Yi CX & Tschöp MH 2012 Brain-gut-adipose-tissue communication pathways at a glance. *Disease Models & Mechanisms* **5** 583–587. (doi:10.1242/dmm.009902)
- Zhang X, Zhang G, Zhang H, Karin M, Bai H & Cai D 2008 Hypothalamic IKK β /NF-kappaB and ER stress link overnutrition to energy imbalance and obesity. *Cell* **135** 61–73. (doi:10.1016/j.cell.2008.07.043)
- Zhang Y, Wu X, He Y, Kastin AJ, Hsueh H, Rosenblum CI & Pan W 2009 Melanocortin potentiates leptin-induced STAT3 signaling via MAPK pathway. *Journal of Neurochemistry* **110** 390–399. (doi:10.1111/j.1471-4159.2009.06144.x)
- Zhu DY, Liu SH, Sun HS & Lu YM 2003 Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates neurogenesis in the adult rodent dentate gyrus. *Journal of Neuroscience* **23** 223–229.
- Zohar M & Salomon Y 1992 Melanocortins stimulate proliferation and induce morphological changes in cultured rat astrocytes by distinct transducing mechanisms. *Brain Research* **576** 49–58. (doi:10.1016/0006-8993(92)90608-C)

Received in final form 27 June 2013

Accepted 23 July 2013

Accepted Preprint published online 23 July 2013