Review

The critical role of adrenomedullin and its binding protein, AMBP-1, in neuroprotection

Cletus Cheyuo¹-³, Weng-Lang Yang¹-³ and Ping Wang¹-³,*

¹ Center for Immunology and Inflammation, The Feinstein Institute for Medical Research, North Shore-LI Jewish Health System, 350 Community Drive, Manhasset, NY 11030, USA
² Elmezzi Graduate School of Molecular Medicine, Manhasset, NY 11030, USA
³ Department of Surgery, Hofstra North Shore-LI School of Medicine, Manhasset, NY 11030, USA
*Corresponding author
e-mail: pwang@nhs.edu

Abstract

Chronic neurodegenerative disorders and acute injuries of the central nervous system exert a prohibitive economic burden, which is aggravated by an unmet medical need for the development of effective neurotherapeutics. The evolutionarily conserved neuropeptide, adrenomedullin (AM), and its binding protein, AMBP-1, also known as complement factor H, play important roles in brain physiology, and their expression is altered in brain pathology. In this review, we discuss the molecular regulation of AM and AMBP-1 and the pivotal roles they play in neuroprotection following brain injury. We assess the reciprocal synergistic effects of AM and AMBP-1 and make suggestions for the design of a novel combination neurotherapy devoid of the potential hypotensive effects of AM while optimizing its neuroprotective property.

Keywords: adrenomedullin; AMBP-1; apoptosis; inflammation; neurodegenerative disease; neuroprotection.

Introduction

Chronic neurodegenerative disorders and acute insults of the central nervous system (CNS), such as stroke, are major causes of mortality and disability worldwide. The collective cost of CNS diseases is staggering, with stroke, the third leading cause of death, estimated at 73.7 billion dollars (Lloyd-Jones et al., 2010), and other neurodegenerative diseases estimated to cost 768 million dollars annually in the USA (Lunn et al., 2011). Superimposed on the prohibitive economic burden is an unmet medical need for the development of effective neurotherapeutics. Neuronal damage, which characterizes CNS diseases, is mediated by complex interrelated molecular mechanisms, which include neuroinflammation, reactive oxygen and nitrosative stress, excitotoxicity, and apoptosis (Moskowitz et al., 2010; Khandelwal et al., 2011). Successful development of CNS therapeutics depends on the identification of target molecules or combination of molecules that can effectively mitigate the pathogenic mechanisms involved in neuronal injury.

Adrenomedullin (AM), a member of the calcitonin gene-related peptide (CGRP) family of proteins (Ogoshi et al., 2006), is an evolutionarily conserved neuropeptide, first isolated from human pheochromocytoma cells (Kitamura et al., 1993) and later found to be widely distributed throughout mammalian tissues, including the brain (Zudaire et al., 2004). AM has shown neuroprotection in experimental brain disease models including ischemic stroke (Xia et al., 2006) and traumatic brain injury (Armstead et al., 2010), as well as cardioprotection in human myocardial infarction (Kataoka et al., 2010). The effects of AM are mediated by the functional receptor combination of calcitonin receptor-like receptor (CRLR) with receptor-activity-modifying protein 2 (RAMP-2) or RAMP-3 (McLatchie et al., 1998). Pio et al. (2001) reported that complement factor H is an AM-binding protein (AMBP-1), which enhances the biological effects of AM. Complement factor H is a negative regulator of the alternative complement pathway (Whaley and Ruddy, 1976), and its activity is reciprocally enhanced by AM (Pio et al., 2001). Complement activation has been reported to be deleterious in many brain diseases, namely stroke (Mocco et al., 2006; Szeplaki et al., 2009), traumatic injury (Sewell et al., 2004; Bellander et al., 2011), and Alzheimer disease (Kolev et al., 2009). Not surprisingly, inhibition of complement activation has been demonstrated to protect against brain damage (Arumugam et al., 2009).

Despite their reported neuroprotection in preclinical studies, neither AM nor AMBP-1 nor their combination has been successfully developed as a CNS therapeutic. This is in part because the mechanisms of action of AM, AMBP-1, or their combination therapy in brain diseases are not well understood. Here we discuss the regulation of AM and AMBP-1 in brain diseases and the mechanisms by which they inhibit the pathologic processes that lead to brain damage. We assess the

---

*P. Wang is an inventor of the pending Patent Cooperation Treaty (PCT) application 60/557, 935, ‘AM and AM binding protein-1 for ischemia/reperfusion treatment’. This patent application covers the fundamental concept of using human AM/AMBP-1 for the treatment of ischemia/reperfusion injury.
therapeutic potential of AM alone, AMBP-1 alone, and the synergistic action of AM with AMBP-1 in neuroprotection. Finally, we make suggestions for further development of AM and AMBP-1 as a novel therapy for brain diseases.

**Access of AM and AMBP-1 to their receptors and substrates in the brain**

To exert their biological effects within the brain, AM and AMBP-1 need to interact with their respective receptors and substrates expressed within the brain.

**AM and CRLR-RAMP-2/3 expression in the brain**

The human AM gene, which is located on chromosome 11, consists of four exons and three introns. The 5′ flanking region of the gene contains TATA, CAAT, and GC boxes as well as response elements for several transcription factors and regulatory proteins. The first intron also contains several response elements for transcriptional regulation. Thus, the production of AM is highly regulated. It has been demonstrated that hypoxia, shear stress, and inflammatory cytokines such as TNF-α and IL-1α can increase AM production, whereas interferon-γ (IFN-γ) and TGF-β1 down-regulate its transcription (Eto et al., 2003). AM is synthesized as part of a larger precursor molecule, termed preproadrenomedullin, which is a 185-amino acid (aa)-polypeptide with an amino terminal 21-aa signal peptide for targeting to the rough endoplasmic reticulum (ER). The signal peptide is cleaved off in ER lumen to yield a 164-aa polypeptide called proadrenomedullin. From the ER through the Golgi apparatus to the secretory granules, proadrenomedullin is sequentially processed by exopeptidases, endopeptidases, and amidating enzymes to yield two active cleavage products; AM, a 52-aa peptide, and proadrenomedullin N-terminal 20 peptide (PAMP), and other nonactive peptides. AM and PAMP are secreted by exocytosis (Martinez et al., 2001; Lopez and Martinez, 2002). The secreted form of AM has an internal six-amino-acid ring structure, formed by a disulfide bond between Cys16 and Cys21 and an amidated C-terminus, which are necessary for its functions (Eguchi et al., 1994). As with most peptide molecules, the biological activity of AM depends on its secondary structure. Using nuclear magnetic resonance (NMR) spectroscopy, Pérez-Castells et al. (2012) recently elucidated the three-dimensional structure of micelle-bound AM (mimicking biological membranes). Micelle-bound AM was reported as consisting of a central α-helical region, spanning residues 22–34, flanked by disordered segments at both N- and C-termini. In contrast, nonmicelle-bound AM, dissolved in water, had a disordered structure, with no α-helical content. Thus, interaction with membranes induces the active three-dimensional structure of AM (Pérez-Castells et al., 2012) (Figure 1).

![Figure 1](image-url)  
**Figure 1**  Illustration of the synthesis and posttranslational modification of AM. The human AM gene consists of four exons (Ex) and three introns, with regulatory elements such as TATA, CAAT, and GC boxes as well as response elements for several regulatory proteins in the 5′ flanking region. The first intron also contains several response elements. The gene encodes 185-aa preproadrenomedullin, containing an amino terminal 21-aa signal peptide (SNP), which targets the polypeptide to the rough ER. The signal peptide is cleaved off in the lumen of the ER to yield a 164-aa polypeptide called proadrenomedullin. Proadrenomedullin is sequentially processed by exopeptidases, endopeptidases, and amidating enzymes in the Golgi apparatus to yield two active cleavage products: AM, a 52-aa peptide, and PAMPs, which are secreted. The secreted form of AM has an internal six-amino-acid ring structure, formed by a disulfide bond between Cys16 and Cys21, and an amidated C-terminus, which are necessary for its functions. Also important for the function of AM is its secondary structure, which consists of a central α-helical region, spanning residues 22–34, flanked by disordered segments at both N- and C-termini. AM mediates its biological effects via its receptor, CRLR in combination with RAMP-2 or RAMP-3.
The critical role of AM in the physiology of the brain is underscored by the fact that it is richly expressed in cerebral vascular endothelial cells (Kis et al., 2002) as well as all the major cell types within the brain, including neurons (Serrano et al., 2008), astrocytes (Takahashi et al., 2000), microglia (Veroni et al., 2010), and oligodendrocytes (Uezono et al., 2001). AM secreted by these cellular sources within the brain is thought to act in an autocrine or paracrine fashion (Tixier et al., 2008). Systemically administered AM needs to cross the blood-brain barrier to mediate beneficial effects in brain diseases. Kastin et al. (2001) reported that intravenously administered radiolabeled AM ([125I]-AM) is able to cross the intact blood-brain barrier. It was determined that the intact AM crosses the blood-brain barrier via a saturable unidirectional blood-to-brain transport system. This transport system does not interact with other members of the CGRP family of proteins.

The AM receptor consists of the seven-transmembrane G-protein-coupled protein, CRLR, which interacts noncovalently with the single-transmembrane proteins RAMP-2 or RAMP-3 to form the functional receptor. The RAMP-2 or RAMP-3 coexpression with CRLR determines the selectivity of the receptor for AM (McLatchie et al., 1998). RAMP-2 and RAMP-3 expression (Ueda et al., 2001) and colocalization with CRLR (Oliver et al., 2002) have been demonstrated in the brain. RAMP-3 is strongly expressed in the thalamic nuclei, layer VI of the neocortex, and the granular layer of the cerebellum, whereas RAMP-2 is expressed in the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala, and Purkinje cell layer of the cerebellum (Oliver et al., 2001). Within these regions of the brain, RAMP-2 and RAMP-3 are expressed in neurons, astrocytes, and vascular endothelial cells (Zimmermann et al., 1996; Oliver et al., 2002; Xu and Krukoff, 2007). These same cell types also express AM, thus forming an autocrine loop for the biological effects of AM (Tixier et al., 2008). In radioligand-binding studies, AM and AMBP-1 first morphological evidence for the potential interaction of AM and AMBP-1 when they demonstrated colocalization of these two proteins in the rat brain. The pivotal role of AMBP-1 in brain physiology is supported by the fact that the CFH Y402H polymorphism of the AMBP-1/complement factor H gene is associated with adverse outcomes in ischemic (Volcik et al., 2008) and hemorrhagic (Appelboom et al., 2011) stroke and age-related macular degeneration (AMD) (Klein et al., 2005).

**AMB-1 and complement expression in the brain**

Complement, an integral part of the innate immune system, has been implicated in the pathogenesis of many neurodegenerative disorders including Alzheimer disease (Kolev et al., 2009), Huntington disease (Singhrao et al., 1999), stroke (Mocco et al., 2006; Szepliki et al., 2009), and Parkinson disease (Loeffler et al., 2006). The liver is the major source of complement proteins in the serum (Alper et al., 1980). However, the discovery of a significant role of complement in many chronic brain diseases and the fact that most serum complement proteins do not cross the intact blood-brain barrier prompted investigations into a local source of complement in the pathogenesis of brain diseases. Studies have revealed that complement proteins are expressed in cerebral endothelial cells (Vastag et al., 1998), astrocytes (Walker et al., 1998), neurons (Shen et al., 1997), and oligodendrocytes (Hosokawa et al., 2003). The complement system consists of soluble and membrane-bound proteins, which when activated can initiate inflammation and tissue injury through the formation of mediators that cause increase in vascular permeability, neutrophil chemotaxis, and cell lysis (Veerhuis et al., 2011). In conditions of health, the brain tissue is protected from the deleterious effects of spontaneous complement activation by a number of complement inhibitors (Veerhuis et al., 2011).

AMB-1, also known as complement factor H, is one of the negative regulators of the complement system (Whaley and Ruddy, 1976). AMBP-1 is a 150-kDa protein that is composed of 20 repetitive domains termed short consensus repeats (SCR), with each SCR being about 60 aa in length (Ripoche et al., 1988). Being a large protein, serum AMBP-1, secreted mainly by the liver, may not be able to cross the intact blood-brain barrier. However, the access of serum AMBP-1 to the brain may be enhanced by the blood-brain barrier disruption associated with certain brain pathologies such as stroke (Belayev et al., 1996). We have recently shown that systemically administered AMBP-1 can enter the brain after ischemic stroke (Chaung et al., 2011). Local AMBP-1 expression in the brain has also been shown in cerebral endothelial cells (Vastag et al., 1998), neurons (Thomas et al., 2000), and astrocytes (Praczk et al., 2011). AMBP-1 regulates the complement cascade by binding to C3b and preventing the formation of the alternative complement pathway C3 convertase, C3bBb (Whaley and Ruddy, 1976). AM and AMBP-1 interact with each other and reciprocally potentiate each other's activity (Pio et al., 2001). Serrano et al. (2003) provided the first morphological evidence for the potential interaction of AM and AMBP-1 *in vivo* when they demonstrated colocalization of these two proteins in the rat brain. The pivotal role of AMBP-1 in brain physiology is supported by the fact that the CFH Y402H polymorphism of the AMBP-1/complement factor H gene is associated with adverse outcomes in ischemic (Volcik et al., 2008) and hemorrhagic (Appelboom et al., 2011) stroke and age-related macular degeneration (AMD) (Klein et al., 2005).

**Regulation of AM and AMBP-1 levels during brain damage**

The essential roles of AM and AMBP-1 in brain physiology imply that alterations in the levels of these proteins during brain pathology will have implications for disease progression and outcomes.

AM **is up-regulated as an adaptive stress response in brain injury**

AM is up-regulated in brain diseases (Serrano et al., 2002), and investigations using loss-of-function and gain-of-function
paradigms have established that the up-regulation of AM under conditions of brain damage is a rescue stress response. In a mouse brain-specific AM knockout model, Fernández et al. (2008) discovered that lack of AM in the brain was associated with tubulin hyperpolymerization and decreased resistance to hypobaric hypoxia. Similarly, Miyamoto et al. (2009) also demonstrated that AM heterozygous knockout led to accumulation of reactive oxygen species and increase in infarct size and neurological deficits after experimental stroke. In contrast, AM overexpression mice were shown to be protected from ischemic stroke (Xia et al., 2006) and traumatic brain injury (Armstead et al., 2010).

Hypoxia-inducible factor 1 (HIF-1) has been implicated in the up-regulation of AM under hypoxic/ischemic stress (Garayoa et al., 2000). HIF-1 is a heterodimeric transcription factor that consists of a β-subunit and an oxygen-sensitive α-subunit (Chan and Giaccia, 2010). Under normoxic conditions, the α-subunit becomes hydroxylated on two conserved proline residues by prolyl hydroxylases. The hydroxylated α-subunit is then recognized by von Hippel-Lindau E3 ubiquitin ligase, which ubiquitinylates it and targets it to the proteasomal degradation pathway. Under hypoxic conditions, the α-subunit does not get hydroxylated. Thus, the α-subunit accumulates and forms the functional heterodimer with the β-subunit, which then translocates into the nucleus to activate the transcription of genes with HIF-1 response element (HRE) (Chan and Giaccia, 2010). Garayoa et al. (2000) discovered several HREs in the human AM promoter, and went further to demonstrate HIF-1-mediated up-regulation of AM under hypoxic conditions (Figure 2). HIF-1 expression has also been shown to increase with age (Leiser and Kaeberlein, 2010). Thus, in the aged population, with increased prevalence of neurodegenerative diseases, HIF-1-mediated increases in AM (Hwang et al., 2007) may also play an adaptive role in limiting neuronal damage. During brain injury, matrix metalloproteinase 2 (MMP-2) is also up-regulated (Bhatt and Addepalli, 2012). Martínez et al. (2004) discovered that MMP-2 degraded AM and that AMBP-1 was essential in preventing MMP-2-mediated degradation of AM.

**Alterations in AMBP-1 levels in brain diseases**

Several studies have reported down-regulation of AMBP-1 in a variety of brain pathologies such as Alzheimer disease (Lukiw et al., 2008), AMD (Chen et al., 2007), and stroke (Chaung et al., 2011). However, in certain CNS pathologies such as multiple sclerosis (Ingram et al., 2010), there is a reported increase in AMBP-1 levels. Inflammatory cytokines, particularly IFN-γ, have been recognized as positive regulators of AMBP-1 expression (Wu et al., 2007b), whereas inflammatory mediators, such as TNF-α and IL-1β, can lead to down-regulation of AMBP-1.

Unauthenticated
Download Date | 6/23/17 8:23 AM
oxidative stress (Lukiw et al., 2008) and NF-κB-sensitive microRNA-146a (Pogue et al., 2009) have been shown to down-regulate AMBP-1. The balance between negative and positive regulators will determine whether AMBP-1 level in brain disease is up- or down-regulated. Wu et al. (2007b) demonstrated that during inflammation, IFN-γ stimulates the nuclear translocation of STAT1 together with relatively small amount of FOXO3 to bind to the STAT1 response element in the AMBP-1 promoter region, leading to up-regulation of AMBP-1 expression. However, in the presence of reactive oxygen species, FOXO3 undergoes a conformational change by acetylation. The acetylated FOXO3 binds to its DNA binding site on the AMBP-1 promoter, instead of the STAT1 site, leading to suppression of AMBP-1 expression (Figure 2). Interestingly, IFN-γ, a positive regulator of AMBP-1, is not appreciably expressed in ischemic stroke (Jander et al., 2002), whereas reactive oxygen species, negative regulators of AMBP-1, are produced in large amounts (Raz et al., 2010). This situation will shift the balance toward down-regulation of AMBP-1 in ischemic stroke. We recently demonstrated a decrease in AMBP-1 expression after ischemic stroke in rats (Chaung et al., 2011). A decrease in AMBP-1 levels in brain injury could lead to overactivation of the alternative complement pathway and increased inflammation.

**Therapeutic applications for AM and AMBP-1 in brain diseases**

Inflammation, oxidative stress, apoptosis, and excitotoxicity have been implicated in the pathogenesis of many neurodegenerative disorders and acute traumatic brain injury (Moosmann et al., 2001; Moskowitz et al., 2010; Khandelwal et al., 2011). Inflammation, which involves tissue infiltration by immune cells and cytokine production, plays a central role in brain damage in Alzheimer disease (Jaworski et al., 2011), Parkinson disease (Swanson et al., 2011), Huntington disease (Khoshnan et al., 2004), stroke (Niu et al., 2012), and traumatic brain injury (Helmy et al., 2011). Inflammatory cells, such as neutrophils, produce reactive oxygen species, which cause membrane damage by lipid peroxidation and further increase inflammation (Carrillo et al., 2011). Apoptotic cell death involves DNA fragmentation caused by activation of the caspase cascade (Kim et al., 2011). Excitotoxicity is triggered by excessive stimulation of N-methyl-d-aspartate receptors by glutamate, ultimately resulting in neuronal injury (Sattler and Tymianski, 2001). AM and AMBP-1 have been used therapeutically to target these molecular mechanisms in several experimental models of brain disease.

**Therapeutic applications of AM in brain disease**

The integrity of the blood-brain barrier is essential for brain function. Blood-brain barrier disruption occurs in conditions of acute brain damage such as stroke (Belavy et al., 1996), leading to increase in brain edema. The blood-brain barrier consists of cerebral endothelial tight junctions and astrocyte foot processes (Abbott et al., 2010). The mechanisms involved in blood-brain barrier breakdown following brain injury include endothelial damage and down-regulation of tight junction proteins (Abbott, 2002). AM has been shown to protect endothelial cells from oxidative damage (Chen et al., 2006). Honda et al. (2006) also reported that AM maintained blood-brain barrier integrity by up-regulating claudin-5, a critical component of the endothelial tight junction. The AM-mediated blood-brain barrier protection may explain, in part, the observation by Kondoh et al. (2011) that AM decreases cerebral edema after focal cerebral ischemia in rats. Another important role of AM in cerebral vascular biology is the maintenance of autoregulation (Armstead et al., 2010). Autoregulation ensures that cerebral perfusion is maintained during disturbances of cerebral blood flow such as in hypovolemia and cerebral ischemia (Aaslid et al., 1989). Thus, failure of autoregulation contributes to cerebral ischemic damage. In a piglet model of fluid percussion injury of the brain, Armstead et al. reported that AM prevented loss of cerebral autoregulation through inhibition of the ERK-MAPK pathway (Armstead et al., 2010) and activation of ATP-dependent K channels (Armstead and Vavilala, 2007). AM has also shown neuroprotection in animal models of stroke through inhibition of inflammation (Miyashita et al., 2006) and apoptosis (Xia et al., 2006). The anti-inflammatory effect of AM may be explained by several signaling mechanisms. Miksa et al. (2007) discovered that AM, acting together with AMBP-1, stimulates pyk2-tyrosine kinase-ERK1/2 pathway, leading to up-regulation of peroxisome proliferator-activated receptor γ, which suppresses inflammation. MacManus et al. (2011) also demonstrated that AM may suppress inflammation by causing the accumulation of HIF-1 via de neddylation of cullin-2 during hypoxia. AM may also contribute to brain repair after damage by stimulating angiogenesis and neurogenesis (Miyashita et al., 2006; Vergano-Vera et al., 2010).

**Therapeutic applications of AMBP-1 in brain disease**

Complement activation plays a significant role in neuroinflammation and brain damage (Mocco et al., 2006). Inhibition of complement has shown protection against stroke (Arumugam et al., 2009), traumatic brain injury (Rancan et al., 2003), and experimental autoimmune encephalomyelitis (EAE) (Griffiths et al., 2009). Using the chronic neuroinflammatory condition of EAE, Griffiths et al. (2009) demonstrated that administration of complement factor H/AMBP-1 protected neurons from complement opsonization, demyelination, axonal injury, and leukocyte infiltration. In addition, complement factor H has also shown therapeutic benefit in AMD, which is characterized by chronic inflammatory damage to the retina (Weissmann et al., 2011). Malondialdehyde, a marker of oxidative stress, contributes to the retinal damage by modifying proteins on the surface of cells, which then become targets of immune cells, which recognize the modified proteins as danger signals (Miller et al., 2011). Weissmann et al. (2011) discovered that the retinoprotective effect of complement factor H in AMD was mediated by the SCR7 domain of complement factor H, which binds malondialdehyde, leading to suppression of inflammation (Figure 2). The tissue-protective...
role of complement factor H/AMBP-1 is further supported by the association of the CFH Y402H polymorphism with poor outcomes in ischemic (Volcik et al., 2008) and hemorrhagic (Appelboom et al., 2011) stroke.

**AM/AMBP-1 combination therapy in brain disease**

The interaction of AM with AMBP-1 has been well characterized. Martinez et al. (2003) discovered that AMBP-1 has two specific binding domains for AM: a high-affinity binding domain located at the carboxy terminal SCR 15–20 region and a low-affinity binding site in the middle of AMBP-1, at SCR 8–11. The exact mechanism by which the binding of AM to these two sites on AMBP-1 results in the reciprocal potentiation of their individual actions, however, remains to be determined. Interestingly, the elucidation of the three-dimensional structure of AM (Pérez-Castells et al., 2012) may permit some speculation as to the nature of the binding of AM to AMBP-1. The central α-helical structure is common to members of the calcitonin gene-related family of proteins and is important for their antimicrobial activity (Allaker et al., 1999; Martinez et al., 2006). Pio et al. (2001) reported that the binding of AM to AMBP-1 decreased antimicrobial activity. Thus, one may speculate that the binding of AM to AMBP-1 directly or indirectly involves the α-helical region, leading to a perturbation in its antimicrobial function, while potentiating other functions.

As AM and AMBP-1 both exert neuroprotective effects and reciprocally potentiate each other’s activity, an attractive novel therapeutic option for brain diseases would be a combination therapy with the two molecules. We recently discovered that the combination of AM with AMBP-1 suppressed inflammation and apoptosis, leading to decreased brain injury in an experimental model of ischemic stroke (Chaung et al., 2011). Wang and Yang (2009) determined that the protective role of the coadministration of AM/AMBP-1 in neuronal hypoxia was mediated by elevation of cyclic AMP and activation of protein kinase A, ultimately leading to inhibition of neuronal apoptosis (Figure 2).

The high mortality associated with brain diseases such as stroke is related in part to a systemic immune response characterized by immunosuppression, which predisposes patients to sepsis. This state of immunosuppression is thought to be mediated by a shift from T helper cell (T_{h}) 1-type to T_{h}2-type cytokine production (Prass et al., 2003; Wong et al., 2011). Attenuation of the systemic immune response and treatment of infections following brain injury lead to improvement in survival (Meisel et al., 2004; Meisel and Meisel, 2011). Interestingly, AM/AMBP-1 combination therapy suppresses cytokine secretion and improves survival in experimental polymicrobial sepsis (Yang et al., 2002; Fowler et al., 2003). In addition, traumatic brain injuries are often complicated by hemorrhagic shock due to bleeding from multiorgan trauma. Wu et al. reported that AM/AMBP-1 treatment maintains cardiovascular stability (Wu et al., 2005) and prevents metabolic acidosis in hemorrhagic shock (Wu et al., 2007a). These systemic effects of AM/AMBP-1 raise the possibility that, in acute brain injury, AM/AMBP-1 treatment may exert local neuroprotective effects in the brain as well as systemic antimicrobial and hemodynamic effects leading to an overall improvement in outcomes. However, the potential beneficial systemic effect of AM/AMBP-1 treatment in acute brain injury has received little attention in the studies of brain diseases. Thus, further research in this area may help to establish the therapeutic potential of AM/AMBP-1 for brain diseases.

**Perspectives and future directions**

AM and its binding protein, AMBP-1, are expressed in the brain and have been shown to play physiological roles. Brain diseases alter the expression of these molecules, and administration of exogenous AM and AMBP-1 protect against brain injury in experimental disease models. conspicuously absent in the studies demonstrating neuroprotective effects of AM and AMBP-1 are dose-response and time-course studies. Investigations on dose-response effects of AM are particularly important given the potential of AM to cause hypotension. Kitamura et al. (1993) established that administration of human AM at a dose of 3 nmol/kg body weight caused a reduction of mean arterial blood pressure by 53±5 mm Hg in rats. Since then, most animal studies have been conducted using AM doses in the nanomolar range. In contrast, a study of AM pharmacokinetics and pharmacodynamics in healthy humans, using far lower doses of AM in the picomolar range, reported a significant decrease in diastolic pressure and a significant increase in pulse rate at an infusion rate of 13.4 pmol/kg/min. In addition, the same study also found a relatively short half-life of 22±1.6 min for circulating AM (Meeran et al., 1997). The hypotensive effects of AM and its relatively short half-life could derail the successful translation of the beneficial neuroprotective effects of AM into human therapy.

Optimistically, the ability of AMBP-1 to potentiate AM’s effects and also prevent its degradation may be useful in designing AM therapies that obviate the hypotensive effects and short half-life. We speculate that low doses of AM in combination with AMBP-1 may produce synergistic neuroprotective effects without adversely decreasing the blood pressure. The protective effect of AMBP-1 on AM degradation could potentially also increase its half-life in a combination therapy. The answers to these speculations will come from comprehensive pharmacokinetic and pharmacodynamic studies of AM/AMBP-1 combination therapy in experimental animal models of brain disease. In addition, time-course studies are also recommended to determine the therapeutic time window of effectiveness of AM/AMBP-1 in acute brain disease such as stroke. A comprehensive assessment of the effect of AM/AMBP-1 treatment on the systemic immune response following acute brain injury will also be useful in establishing the mechanisms involved in tissue protection and overall disease outcome.

In summary, AM and AMBP-1, administered individually or in combination, exert neuroprotective effects in animal models of acute brain injury as well as chronic neurodegenerative diseases. However, extensive preclinical testing remains to be done to determine the appropriate doses, therapeutic.
window, and mechanisms of action. In conclusion, AM and its binding protein, AMBP-1, may be further developed into novel treatment for human brain diseases.

Acknowledgments

This work was supported by NIH grants R01HL076179 and R01GM053008.

References

Ingram, G., Hakobyan, S., Hirst, C.L., Harris, C.L., Pickersgill, T.P., Cossum, M.D., Loveless, S., Robertson, N.P., and Morgan, B.P.


Received January 6, 2012; accepted February 7, 2012

Cletus Cheyuo obtained his MD from the University of Ghana Medical School, Accra, Ghana in 2005. Dr. Cheyuo was offered a position at Bronx-Lebanon Hospital, New York, USA, as a surgical resident in 2008. In 2009, he took time off from his surgery program to pursue a PhD in molecular biology/neuroscience at the Elmezzi Graduate School of Molecular Medicine, and The Feinstein Institute for Medical Research, USA. His current research is on the mechanisms of neuroprotection and neurogenesis following cerebral ischemia. Dr. Cheyuo’s research was recognized nationally in 2010 when he was awarded the New Investigator Award by the American Shock Society for his discovery of ghrelin’s neuroprotective role via the vagus nerve after cerebral ischemia.

Weng-Lang Yang received his PhD in food biochemistry from Rutgers, The State University of New Jersey, USA in 1996. He had a post-doctoral training at The Cancer Institute of New Jersey and became an Assistant Professor of Surgery at the Albert Einstein College of Medicine, New York in 2002. Now, he is an Assistant Professor of Surgery at the Hofstra North Shore-LIJ School of Medicine and Assistant Investigator at The Feinstein Institute for Medical Research, New York. His early research interest is to study cellular responses of solid tumors subjected to surgical ablation and bone morphogenetic protein signaling pathway in regulating cancer metastasis. Currently, his research focuses on development of therapeutic agents for treating organ failure from ischemia/reperfusion injury.
Ping Wang earned his MD from Changwei Medical College, China in 1982 and MS in Surgery from The Third Military Medical University, China in 1985, and received MA ad eundem from Brown University in 1998. He had postdoctoral training at University of Washington School of Medicine, and was on faculty (Assistant Professor to Full Professor) at Michigan State University College of Medicine, Brown University School of Medicine, University of Alabama at Birmingham School of Medicine, and Albert Einstein College of Medicine. He is currently Professor of Surgery and Molecular Medicine, Hofstra North Shore-LIJ School of Medicine and Vice Chairman for Research at the Department of Surgery, North Shore University Hospital and Long Island Jewish Medical Center. He is also an Investigator at the Feinstein Institute for Medical Research. He has been continually NIH-funded Principal Investigator since 1995. His research interests are related to cellular and molecular mechanisms of tissue injury in trauma, hemorrhage, ischemia-reperfusion, and sepsis; novel inflammatory mediators; and drug discovery and preclinical development.