Review

Adrenomedullin, an Autocrine Mediator of Blood-Brain Barrier Function

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Since the discovery that adrenomedullin gene expression is 20- to 40-fold higher in endothelial cells than even in the adrenal medulla, this peptide has been regarded as an important secretory product of the vascular endothelium, together with nitric oxide, eicosanoids, endothelin-1, and other vasoactive metabolites. Cerebral endothelial cells secrete an exceptionally large amount of adrenomedullin, and the adrenomedullin concentration is about 50% higher in the cerebral circulation than in the peripheral vasculature. The adrenomedullin production of cerebral endothelial cells is induced by astrocyte-derived factors. Adrenomedullin causes vasodilation in the cerebral circulation, may participate in the maintenance of the resting cerebral blood flow, and may be protective against ischemic brain injury. Recent data from our laboratory indicate that adrenomedullin, as an endothelium-derived autocrine/paracrine hormone, plays an important role in the regulation of specific blood-brain barrier properties. Adrenomedullin is suggested to be one of the physiological links between astrocyte-derived factors, cyclic adenosine 3′,5′-monophosphate (cAMP), and the induction and maintenance of the blood-brain barrier. Moreover, the role of adrenomedullin in the differentiation and proliferation of endothelial cells and in angiogenesis suggests a more complex function for adrenomedullin in the cerebral circulation and in the development of the blood-brain barrier. (*Hypertens Res* 2003; 26 (Suppl): S61−S70)

***Key Words*: adrenomedullin, blood-brain barrier, cerebral circulation, endothelial cells**

Introduction

Adrenomedullin (AM) was originally isolated from human pheochromocytoma, and the initial reports suggested that the adrenal medulla, ventricle, kidney and lung have the highest levels of expression of AM mRNA (*1*, *2*). However, since the discovery that the AM gene expression is 20- to 40-fold higher in endothelial cells than even in the adrenal medulla (*3*), this peptide has been regarded as an important secretory product of the vascular endothelium, together with nitric oxide (NO), endothelin-1, and other vasoactive metabolites.

Among the endothelial cells of different tissues, the endothelium of the brain is highly specific. Although cerebral endothelial cells (CECs) share many common properties of the peripheral endothelium (*4*), they have a unique morphological and functional feature, the formation of the blood-brain barrier (BBB). The BBB contributes to the stability of the brain parenchymal microenvironment by strictly controlling the traffic of molecules and cells between the blood and

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the central nervous system (*4*, *5*). Recently we described that the AM production of CECs is about one magnitude higher than that of other endothelial cells (*6*). We also found that the AM concentration in the cerebral circulation is significantly higher than that in other tested parts of the circulation (*6*). Moreover, AM has some important functions in the regulation of the BBB (*7*). In this review, we describe current knowledge about the possible role of AM in the cerebral circulation, with a focus on CECs and the BBB.

The Blood-Brain Barrier—An Overview

The BBB is a functional unit of cells forming a dynamic interface between blood and the central nervous system (*4*) and its primary role is to provide special ionic homeostasis for the proper functioning of neural synapses. Tight intercellular junctions between CECs restrict the free passage of molecules and cells from blood to the central nervous system. Glial endfeet cover brain microvessels and capillaries, and both CECs and astrocytes interact with pericytes embedded in the capillary basement membrane, with perivascular microglia and with neurons innervating the cerebral microvessels.

Cells of the cerebral endothelium, like other endothelial cells, produce pro- and anticoagulant proteins (for review see Ref. *4*) as well as vasoactive mediators such as endothelin-1 (*8*), NO (*9*), angiotensin peptides (*10*), AM (*6*), and eicosanoids (*11*, *12*). Endothelial scavenger receptors participate in the metabolism of low density lipoproteins (*13*).

To fulfill the barrier function, CECs, in addition to the above-mentioned general endothelial features, have special, epithelial characteristics which distinguish them from cells of the peripheral endothelium (*4*). Tight intercellular junctions limit paracellular flux, while the small number of pinocytotic vesicles restricts transendothelial traffic of cells and molecules. Accordingly, the electrical resistance of brain capillaries *in vivo* can reach 2,000 Ω (*14*). Tight junctions also contribute to the polarity of CECs, *i.e*., different proteins are expressed on the luminal (*e.g*., P-glycoprotein (*15*)) and on the basal membrane (*e.g*., Na^+/K^+ATPase (*16*)).

To provide substrates to the cells of the nervous system, CECs actively transport nutrients to the brain (see for review: Ref. *17*). Special transporter systems have also been described in the brain endothelium, such as glucose transporter-1 (*18*), amino acid transporters (*19*), or transferrin receptors (*20*).

Recently, efflux transporters have been discovered on CECs; these transporters prevent toxic compounds from entering the central nervous system, and eliminate and/or regulate the interstitial levels of mediators (see for review: Refs. *21* and *22*). The ligands of the adenosine triphosphate-binding casette (ABC)-transporter P-glycoprotein are primarily xenobiotics (*15*), while multidrug resistance proteins pump leukotrienes, and gluthatione conjugated compounds to the blood, whereas other BBB efflux pumps thus far identified help to eliminate organic anions and organic acids (*21*, *22*). Neurotransmitters such as glutamate (*23*), serotonin (*24*), or histamine (*25*) also have specific removal systems at the BBB.

Enzymes specific for the cerebral but not for the peripheral endothelium, such as γ-glutamyl transpeptidase, butyryl choline and acetyl choline esterases, monoamine oxidases A and B, and glutamate decarboxilase, participate in transport processes and formation of the enzymatic BBB (see for review: Ref. *4*).

The BBB phenotype of CECs is induced by the astroglial environment (*26*). Although the effects of neurons on the induction of some specific CEC properties have been described (for review see Ref. *27*), the effect of astroglia on CECs *in vitro* has been more extensively studied. Several BBB parameters are upregulated when CECs are co-cultured with astroglia or cultured in astrocyte-conditioned medium. The expression and junctional localization of the tight junction proteins occludin, claudin-1, and ZO-1 are increased, as are the number of tight junction strands (*28*) and the transendothelial electrical resistance (*17*, *29*, *30*). Astroglia also augment the expression and activity of specific BBB transporters and enzymes (*17*, *29*). The endothelial expression and activity of antioxidant enzymes such as manganese superoxide dismutase are also induced by the astroglia (*31*). Moreover, astrocytes can induce CECs to produce vasoactive mediators such as NO (*32*) or adrenomedullin (*6*), and can induce the expression of vascular endothelial growth factor and angiopoietin receptors on CEC (*33*). Despite the large number of studies, the molecular mechanism of astrocyte signalling to CECs, as well as the related membrane interactions and/or secreted factors, is still an enigma (*5*).

Brain endothelial cells also exert important effects on astroglia, such as induction of astrocyte differentiation, demonstrating that there is cross-talk between the two neighboring cell types (*34*). These results indicate that CECs and astrocytes form a functional unit during the development and maintenance of the BBB.

AM Production in the Cerebral Microcirculation

The finding of the high AM production in peripheral endothelial cells (*3*, *35*) indicates that CECs are probably an important source of AM in the cerebral microcirculation. However, the initial report by Sugo *et al*. (*3*) did not support this hypothesis: these authors found very low AM production by cultured bovine CECs, which was only a few percent of the AM production of rat aortic endothelial cells. Moreover, this observation about the low AM production of CECs was recently supported by Ladoux and Frelin (*36*), who described weak AM mRNA expression in clones of rat CECs by Northern blot analysis. It should be mentioned, however, that CECs passaged a large number of times (10−20 passages) in both studies (*3*, *36*), which might have deteriorated

the original phenotype of the primary cells (*37–39*).

In contrast to these experiments, we have recently found unexpectedly high AM production in primary cultures of rat CECs both at the peptide and at the mRNA levels (*6*). Rat CECs had about one magnitude higher AM production than those reported for other primary cells (for review see Ref. *40*). Only the AM productions of Hs68 and NHLF human fibroblast cells (*41*) and T98G glioblastoma cells (*42*) were close to that of rat CECs; however these are cell lines and not primary cells. Thus the available data indicate that rat CECs have the highest rates of AM synthesis and secretion among the cells studied.

The high AM production of rat CECs was further enhanced by astrocyte-derived factors; significantly elevated AM production was detected in the culture medium of primary rat CECs co-cultured with astrocytes or cultured in astrocyte-conditioned medium (*6*). These results suggest that the *in vivo* AM production may be even higher. AM production by rat CECs, however, could not be induced by cytokines, bacterial lipopolysaccharide, or thrombin (*6*), which are the most powerful inducers of AM release in peripheral endothelial cells (*35*). Our studies also revealed that AM is secreted primarily but not exclusively at the luminal (blood) side of CEC monolayers (*6*). In contrast to AM, endothelin-1, a vasoconstrictor peptide, is secreted mostly toward the abluminal (brain) side of bovine CECs (*43*).

We could not detect AM immunoreactivity in brain microvessels (*6*), whereas Serrano *et al*. (*44*, *45*) found only small immunoreactive deposits in some cerebral capillaries. The weak immunoreactivity in brain endothelial cells and the high, and time-dependently increased level of immunoreactive (ir)-AM in the culture medium (*6*) suggest that most of the AM formed by rat CECs is immediately secreted, as is the case in peripheral endothelial cells (*3*, *35*), vascular smooth muscle cells (*46*) and fibroblasts (*41*). These observations also suggest that immunochemistry may not be the optimal method for studying AM production by the cerebral endothelium.

In addition to the CECs, other cellular elements of the cerebral vessels can produce AM, *i.e.* vascular smooth muscle cells (*3*, *35*), pericytes (*6*), or even astrocytes (*6*, *47*) and neurons (*44*, *48*). All of the above-mentioned cells, however, are on the brain side of the BBB, and thus their contribution to the AM level measured in the cerebral circulation is questionable. AM production by both these cells and the choroid plexus (*49*, *50*) seems more likely to contribute to the AM level of the cerebrospinal fluid (CSF). The AM concentration in the CSF is comparable to (*49*) or lower than (*51*) that in plasma, and it has been suggested that these two compartments regulate AM level independently (*51*, *52*).

In vivo, we found an approximately 50% higher AM concentration in the jugular vein than in the carotid artery (*6*). We also found an approximately 50% higher AM concentration in the venous plasma effluxed from the brain than in the venous plasma from peripheral organs (*6*). These observations strongly support our *in vitro* findings. Previously, no significant difference was observed among ir-AM levels in the venous blood from the kidney, lung, adrenal gland and systemic arterial blood of rats (*53*). However, a significantly lower concentration in the left ventricle and aorta compared to venous side of circulation was reported in some studies and it was also proved that the lung is the major site of AM clearance in humans (*54*, *55*).

We can conclude that the cerebral circulation has an exceptionally high AM concentration due to the significantly elevated basal AM secretion by brain endothelial cells, which is induced by astrocyte-derived factors.

AM Receptors in the Cerebral Microcirculation

When injected intravenously, AM acts predominantly in organs in which the AM gene is highly expressed (*56*). This observation suggests that AM is a local autocrine and/or paracrine hormone (*56*) and that AM released by rat CECs may act primarily on AM receptors present in the cerebral endothelium itself and their neighboring cells.

Three receptors have been proposed to have specific AM-binding properties: L1, which is a putative rat AM receptor (*57*), and combinations of the calcitonin receptor-like receptor (CRLR) with either receptor-activity modifying protein 2 (RAMP-2) or RAMP-3 (*58*). On the other hand, the combination of CRLR with RAMP-1 forms the calcitonin gene-related peptide receptor 1 (CGRP1) (*58*). It seems probable that CRLR–RAMP-2 and CRLR–RAMP-3 compose functional AM receptors, whereas the identity of both human (*59*) and rat L1 (*57*) orphan receptors as AM receptors has been questioned (*60*). We previously characterized the expression of CRLR and RAMP-1, -2, and -3 on isolated cerebral microvessels (*61*), rat CECs and rat cerebral pericytes (*6*) by reverse transcriptase-polymerase chain reaction (RT-PCR), and measured the intracellular cyclic adenosine 3 ,5 - monophosphate (cAMP) concentration after exogenous AM administration. RAMP-2 showed the highest expression, followed by RAMP-3 and RAMP-1, and exogenous AM increased the intracellular cAMP concentration in rat CECs and pericytes, suggesting the existence of functional AM receptors on these cells (*6*). Although astrocyte-derived factors increased the AM production of rat CECs, they did not change the expression of AM receptor components in rat CECs (*6*). Oliver *et al*. (*62*) reported the same expression pattern of RAMPs in the human cerebral vasculature.

There has been no previous publication describing each of the known AM receptor components at the BBB. Moreno *et al*. (*63*) demonstrated the expression of CRLR, as they called the A-CGRP1 receptor, on human CECs, but they did not check the expression of RAMPs. Ladoux and Frelin (*36*) found variable expression of CRLR and RAMPs in 13 clones of cultured rat CECs. However, no significant effect of AM or CGRP on cAMP formation was found (*36*), indicating

that these cloned rat CECs with high passage numbers had no functional AM or CGRP receptors. The presence of specific AM receptors and of an AM-stimulated increase in the level of cAMP in astrocytes has also been reported (*64*).

AM and BBB Functions

cAMP has long been known to contribute to the regulation of BBB functions; for example, cAMP elevates transendothelial electrical resistance and decreases paracellular permeability (*5*, *28*, *30*, *65*), reduces the rate of fluid-phase endocytosis (*30*), and increases P-glycoprotein function (*66*).

Activation of adenylate cyclase is a common consequence of AM receptor activation in a wide variety of cells (*56*). Cultured rat CECs secrete an exceptionally high amount of AM (*6*), and both isolated cerebral microvessels (*61*) and cultured rat CECs (*6*) express mRNA of functional AM receptor components and exhibit a dose-dependent increase in cAMP concentration after administration of exogenous AM. These results and the binding of AM to mouse cerebral capillaries (*67*) suggest that AM plays a role at the BBB.

In a recent study, we provided evidence that AM has cAMP-like effects on specific BBB functions *in vitro* (*7*). Exogenous AM increased transendothelial electrical resistance and reduced endothelial permeability for the low molecular weight sodium fluorescein, which suggests a tightening of intercellular junctions. AM also decreased endothelial fluid phase endocytosis and activated the P-glycoprotein efflux pump in cultures of rat CECs (*7*). Treatment with both the AM receptor antagonist, $AM_{22\text{-}52}$, and the AM antisense oligonucleotide decreased the basal intracellular cAMP level in rat CECs (*6*). Michibata *et al*. (*68*) have reported that neutralization of endogenous AM by monoclonal antibodies reduced the basal cAMP production in bovine aortic endothelial cells, but not in smooth muscle cells. We have also shown that antisense treatment significantly reduces the AM production in primary rat CECs and decreases transendothelial electrical resistance (*7*). It is remarkable that the basal intracellular cAMP concentration is the highest in rat CECs followed by GP8 immortalized rat CECs and human umbilical vein endothelial cells, which corresponds to the AM production of these cells (*6*). These observations suggest that AM, as an autocrine mediator, plays an important role in the maintenance of basal intra-endothelial cAMP level, and that AM is an autocrine inducer of BBB functions of CECs *via* the activation of adenylate cyclase enzyme. Moreover, astrocyte-derived factors have been shown to increase the AM production by primary rat CECs, suggesting that AM is involved in the astrocytic regulation of the BBB phenotype (*5*, *26*). Therefore, AM may constitute a physiological link between astrocyte-derived factors, cAMP, and the induction and maintenance of the BBB properties by rat CECs.

In addition to its role in the regulation of CEC permeability, cAMP is also important in the regulation of the permeability of other endothelial and epithelial cells of the body (*69*). Interestingly, AM knockout homozygous mice die at midgestation with extreme *hydrops fetalis* and cardiovascular abnormalities, including severe hemorrhages and pericardial effusions (*70*, *71*). This suggests a more general role for AM as an endothelium-derived autocrine hormone in the regulation of endothelial permeability.

The Role of AM in the Cerebral Circulation

Several studies have provided evidence that AM has a vasodilatory effect in the cerebral circulation (for review see Ref. *72*). It has been reported that canine basilar arteries showed greater sensitivity to AM than did renal, coronary or femoral arteries (*73*). Robust vasodilator responses to AM have been observed in the cerebral arteries of dogs (*73–75*), rats (*75*, *76*), and humans (*77*), as well as in rat cerebral arterioles (*78*, *79*). Moreover, AM has been shown to induce increases in cerebral (*80*, *81*) and vertebral (*74*) blood flow.

Until recently, the mechanism of action of AM in the cerebral vessels has been poorly defined. It is now clear that the major effect on AM-stimulated cells is an elevation of intracellular cAMP concentration (for review see Ref. *56*). In addition, AM has been reported to activate constitutive NO-synthase by increasing the intracellular calcium concentration in peripheral endothelial cells (*82*, *83*), and NO produced by endothelial cells was shown to contribute to the vasodilator effect of AM *in vivo* (*82*). Therefore, the vasodilator effects of AM may be dependent on at least two mechanisms, a direct action of AM on vascular smooth muscle cells (VSMCs) coupled to the accumulation of intracellular cAMP (*84*), and an indirect mechanism involving the stimulation of NO production (*82*). However, in rat cerebral arterioles, Lang *et al*. (*78*) demonstrated that both ATP-sensitive and calcium-dependent potassium channels play a role in the dilator response to AM.

Pericytes, the capillary counterparts of vascular smooth muscle cells, express AM receptors (*6*). Because of their close proximity to endothelial cells, and their large number in brain capillaries (*85*), cerebral pericytes are putative paracrine targets for AM produced by the cerebral endothelium. Pericytes contain α-smooth muscle actin (*86*), and thus can induce vasoconstriction and vasodilation within capillary beds (*87*). AM may regulate the capillary blood flow acting on pericytes (*43*, *85*), and this mechanism may contribute to the increased cerebral blood flow after exogenous AM administration (*80*).

The observations that AM is secreted in large amounts by the brain endothelium (*6*), that there is a high concentration of AM in the cerebral circulation (*6*), and that AM receptors are expressed in the cerebral vasculature (*6*, *36*, *61–63*, *77*) suggest that AM might play roles in maintaining the resting tone of cerebral vessels and physiologically regulating the cerebral blood flow.

The Role of AM in Cerebral Pathologies

AM and Cerebral Ischemia

Because AM is a cerebral vasodilator, it would be expected that excess production of AM might lead to an improved postischemic neurological outcome. Dogan *et al*. (*80*) and Watanabe *et al*. (*88*) reported beneficial effects of intravenous administration of AM in focal cerebral ischemia in rats. AM tended to suppress the reduction in regional cerebral blood flow after middle cerebral artery occlusion, and inhibited the increase in myeloperoxidase activity (*i.e.*, decreased the number of infiltrating neutrophils) in the ischemic area, which led to significantly decreased brain injury (*80*, *88*). These results are very similar to the data reported for CGRP administration (*89*). On the other hand, intracerebroventricular administration of a high dose of AM before and after occlusion of the middle cerebral artery has been shown to cause a significant increase in the degree of focal cerebral injury (*75*).

Hypoxia increases AM production in cultured rat cerebral (*36*), bovine carotid (*90*), and human coronary artery endothelial cells (*91*). Serrano *et al*. (*45*) demonstrated an increase in AM expression in cortical neurons and in perivascular structures that may represent glial elements or pericytes as well as in endothelial cells. There are several mechanisms by which ischemia may increase AM expression. One of them involves the hypoxia inducible factor-1 (HIF-1) which binds to the DNA motifs known as hypoxia-responsive elements and influences gene expression (*92*). Several hypoxia-responsive elements have been found in human and mouse AM genes (*93*). Another mechanism for increased AM expression could be the augmentation of AM mRNA stability that takes place during hypoxia (*36*, *93*). Other hypoxia-inducible gene products (*94*) at the cerebral endothelium may include autocrine and paracrine regulators of BBB permeability and vasoreactivity (*e.g.*, AM, endothelin-1, NO, plasminogen activator inhibitor-1, vascular endothelial growth factor receptor), transporters (*e.g.*, glucose transporter-1, transferrin receptor), and the P-glycoprotein efflux pump. Exogenous AM administration has been shown to increase the barrier phenotype in monolayers of CECs (*7*), which may suggest that increased AM production can play a protective role in the maintenance of BBB integrity during hypoxia.

The cytoprotective effect of AM in the cardiovascular and renal system has been well documented (*95–97*). Serrano *et al*. (*45*) reported a correlation between AM expression and the degree of structural conservation of the cortical neurons after oxygen-glucose deprivation. The high expression of AM observed after 10–12 h of reperfusion may have contributed to the preservation of normal morphology in the immunopositive cortical neurons, suggesting a neuroprotective role for AM (*45*).

AM and Subarachnoid Hemorrhage

AM is suspected to play a role in the pathologic mechanism of subarachnoid hemorrhage (SAH). Two investigations have reported that plasma concentrations of AM were increased in patients suffering from SAH throughout the study period (*52*, *98*), and in the latter study the AM levels were correlated with the clinical condition of patients (*98*). However, in these studies no relationship was found between plasma AM concentration and the onset of cerebral vasospasm (*52*, *98*), a major cause of delayed brain ischemia after SAH (*99*). On the other hand, Wijdicks *et al*. (*100*) reported a significant correlation between increased levels of circulating AM and the presence of vasospasm. In another study, patients with symptomatic vasospasm were found to have significantly higher levels of AM in the cerebrospinal fluid (CSF) than those without vasospasm, the concentration of AM in the CSF increased with time in response to brain ischemia, and the increase was unrelated to the plasma concentrations (*52*). It has been speculated that the elevated plasma AM concentrations may be the consequence of increased sympathetic activity after SAH (*101*), which may stimulate vascular tissue to secrete AM into the bloodstream (*102*). The increased concentration of AM in the CSF may be the result of increased AM production by the ischemic brain tissue (*36*, *45*, *75*), or may be the result of BBB disruption (*103*, *104*), which can lead to AM flux from the cerebral blood to the CSF. The relationship between AM and SAH needs to be explored further in the laboratory and in a larger series of patients with SAH.

AM and Migraine

Migraine is one of the most common neurological disorders. The pathophysiology of migraine is still not completely understood, but there is a clear association between head pain and the release of CGRP. CGRP levels are increased in the circulation (*105*) and saliva (*106*) during migraine attacks and in migraine sufferers outside of attacks (*107*). CGRP is contained in nociceptive afferent C and Aδ fibers innervating cerebral vessels (*108*). These perivascular fibers release CGRP and dilate cerebral blood vessels acting on CGRP receptors, thereby resulting in the exacerbation of headache pain. Cerebral vessels are pharmacologically classified as possessing CGRP1 receptors. Recent studies (*6*, *62*) have shown that CGRP receptors are present in vascular smooth muscle cells but not in CECs, supporting a minor role of endothelial cells in mediating vasomotility in response to CGRP. Although, at the present time, there is no direct evidence that AM is involved in the pathogenesis of migraine, several facts should be considered in this regard: 1) AM and CGRP can act on the same receptors (*58*) and have similar modes of action (*109*); 2) CGRP is present in perivascular sensory nerve fibers but not expressed in CECs (*36*), while AM is not present in perivascular nerves (*44*, *77*) but is se-

creted in large amounts by the brain endothelium (*6*); 3) CGRP has no significant role in the maintenance of resting tone of cerebral vessels (*110*) but is responsible for adjusting local cerebral blood flow in response to nociceptive signals (*77*, *111*), while AM might be important for maintaining the resting tone of cerebral vessels; and 4) AM presynaptically inhibits neurotransmission in the perivascular CGRPergic nerves of rat mesenteric resistance arteries, probably decreasing CGRP release (*112*).

Conclusions

AM has been shown to have a wide range of effects on circulation, including vasodilation (*113*), regulation of vascular smooth muscle cell proliferation (*114*), inhibition of endothelial apoptosis (*115–117*), promotion of angiogenesis (*118*), and regulation of blood coagulation and fibrinolysis (*119*). The results of studies using transgenic overexpressing and knockout models have further emphasized that AM is crucial to vascular morphogenesis and function (*70*, *71*, *97*, *120*). The circulating AM plasma concentration has been reported to be elevated in a variety of conditions affecting the cardiovascular system, including essential hypertension (*121*), chronic heart failure (*122*), diabetes (*123*, *124*), sepsis (*125*), and normal pregnancy (*126*). Because such a broad range of conditions have been associated with AM elevation, it seems likely that increases in AM are not causative of disease but rather compensatory to other cardiovascular events. The results on transgenic mice overexpressing the AM gene support the idea of a protective role for AM (*97*).

The AM system may be especially important in the cerebral circulation. The concentration of AM is about 50% higher here than in other regional circulations due to an astrocyte-induced elevation of the AM production by CECs (*6*). AM causes vasodilation in the cerebral circulation and may be important in the maintenance of the resting cerebral blood flow and protective against ischemic brain injury. Recent data from our laboratory indicate that AM, as an endothelium-derived autocrine/paracrine hormone, plays an important role in the regulation of specific BBB properties (*7*). AM may be one of the physiological links between astrocyte-derived factors, cAMP, and the induction and maintenance of the BBB. Moreover, the role of AM in the differentiation and proliferation of peripheral endothelial cells (*117*) and in angiogenesis (*118*) suggests a more complex function for AM in the cerebral circulation and in the development of BBB.

The characteristics and functions of AM and CGRP receptors will require further investigation. In particular, there is need for the development of novel, potent, specific and possibly non-peptide receptor antagonists as potential therapeutic tools for the suppression of AM-mediated proliferative effects in tumours or as anti-migraine drugs (*127*). Similarly, the development of non-peptide agonists may be useful in providing a protective effect in ischemic brain injury and in brain edema.

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