DANISH MEDICAL JOURNAL

Invited State-Of-The-Art Review

Adrenomedullin in pulmonary hypertension

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Dan Med J 2024;71(12):A07240498. doi: 10.61409/A07240498

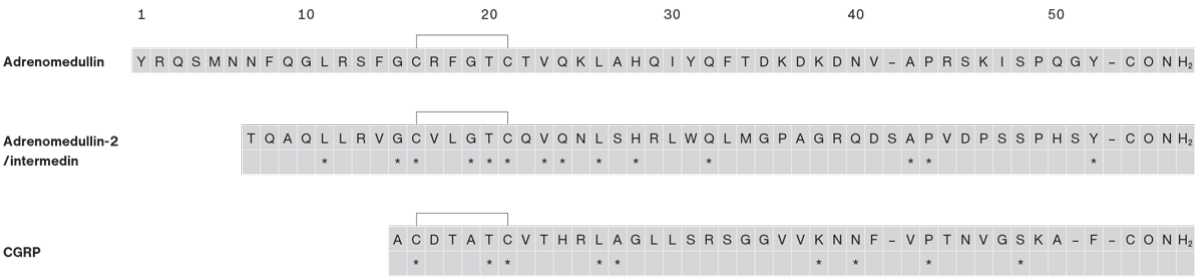
ABSTRACT

Adrenomedullin (AM) exerts strong pulmonary vasodilatory effects. These effects are mediated in part by nitric oxide. Plasma AM levels are increased in patients with pulmonary hypertension and correlate with disease severity and poor outcomes. Acute administration of AM improves the haemodynamics in patients with pulmonary hypertension, while chronic administration prevents the onset of pulmonary hypertension in animal models and delays its progression. Thus, AM is closely related to the pathophysiology of pulmonary hypertension and may be a promising therapeutic target.

KEY POINTS

- Adrenomedullin (AM) exhibits strong vasodilatory activity in the pulmonary circulation where AM receptors are highly expressed.
- In patients with pulmonary hypertension, plasma AM levels are increased in proportion to the pulmonary haemodynamics and disease severity, making plasma AM levels a potential prognostic indicator.
- Long-term AM administration ameliorates the progression of pulmonary vascular remodelling as a result of its antiproliferative, antimigration, antifibrotic, antioxidative and anti-inflammatory effects.

Various neurohumoral factors contribute to regulating pulmonary circulation and the pathophysiology of pulmonary hypertension (PH) [1]. Among these is adrenomedullin (AM), a potent, long-lasting, vasodilatory peptide originally discovered in human pheochromocytoma tissue [2]. AM consists of 52 amino acids with an intramolecular disulfide bond and shares slight homology with calcitonin gene-related peptide (CGRP) (**Figure 1**). AM expression is widely distributed in the cardiovascular system, including both vascular endothelial and smooth muscle cells [3, 4]. In addition, AM and its receptor components, which include calcitonin receptor-like receptor and receptor activity-modifying protein (RAMP)2 and RAMP3 [5], co-localise in those regions, suggesting that AM acts in an autocrine and/or paracrine fashion to exert its effects on the pulmonary vasculature [6]. The actions of AM include inhibitory effects on the proliferation and migration of vascular smooth muscle cells and collagen production by fibroblasts [7, 8]. This suggests that AM acts as an anti-proliferative, anti-migrative and anti-fibrotic factor within the pulmonary vasculature [9, 10]. In addition, AM has been reported to exert anti-apoptotic, angiogenic, anti-inflammatory and antioxidant effects [11, 12]. Therefore, AM is thought to play a protective role against the development of PH. Here, we describe the significance of AM in pulmonary circulation and PH.



*) Sequence identity with adrenomedullin.

FIGURE 1 Comparison of the amino acid sequences of human adrenomedullin, adrenomedullin-2/intermedin and calcitonin gene-related peptide (CGRP).

Pharmacological action of adrenomedullin in pulmonary circulation

Vasodilatory activity of adrenomedullin in the pulmonary circulation

Soon after the discovery of the AM peptide, its vasorelaxant activity in the pulmonary vascular bed was observed in cats [13]. Administration of AM significantly and dose-dependently decreased lobar pulmonary arterial pressure (PAP). AM and AM(15-52) exhibited similar vasodilator activity, suggesting that amino acids(15-52) in the AM are important for vasodilator activity in the pulmonary vascular bed of cats. When the pulmonary vascular responses to AM were compared with those of CGRP, it was found that intralobar injections of AM or CGRP dose-dependently decreased lobar arterial pressure while only slightly reducing systemic arterial pressure [14]. This finding confirmed that AM and CGRP both exert vasodilator activity in the pulmonary vascular bed.

The mechanism by which AM induces pulmonary vasodilation was first investigated using blood-perfused rat lungs [15]. It was observed that the pulmonary vasodilatory effect of AM was unaffected by an inhibitor of prostaglandin E, nitric oxide or 5-HT, antagonists of adenosine receptor or CGRP, or thromboxane A2 mimic. AM was therefore reported to dilate the pulmonary vasculature independently of those known cascades. It has also been reported that the pulmonary vasodilatory response to human AM was more potent in cats than in rats, whereas the response to human CGRP was similar in rats and cats [16]. Moreover, after administration of L-NAME, an endothelial nitric oxide synthase inhibitor, pulmonary vasodilator responses to AM, AM(15-52) and CGRP were all significantly decreased in rats but not cats. Thus, pulmonary vascular responses to AM are likely mediated by different mechanisms in different species [17]. Using an X-ray television system to examine cat lungs, AM induced marked vasodilation in small pulmonary arteries, with the greatest dilation occurring in peripheral arterial segments with internal diameters of 100-500 microns. Conversely, AM had much less effect in small veins. The CGRP antagonist CGRP(8-37) had no significant inhibitory effect on the AM-induced increases in internal arterial diameters, suggesting that AM-induced pulmonary vasodilation is mediated by its own specific receptor [18].

Vasodilatory action of adrenomedullin under hypoxic conditions

Under normoxic conditions, AM and AM(13-52) dilated precontracted pulmonary arterial rings from rats, and the effect was abolished by L-NAME. Under hypoxic conditions, AM(13-52) failed to relax pulmonary arterial rings, whereas AM elicited modest relaxation, and that effect was abolished by indomethacin. Amino acids 1-12 thus appear to contribute to the vasodilatory effect of AM in hypoxic pulmonary arteries, and the effect is mediated via an indomethacin-sensitive pathway [19]. Both AM and CGRP dose-dependently decreased PAP in a rat hypoxia-induced PH model, and pretreatment with CGRP(8-37) significantly reduced the hypotensive response to AM. This suggests that by acting via the CGRP receptor, AM may be useful for acute pharmacological manipulation of PAP in hypoxia-induced PH [20]. AM-2/intermedin (Figure 1), a member of the CGRP family

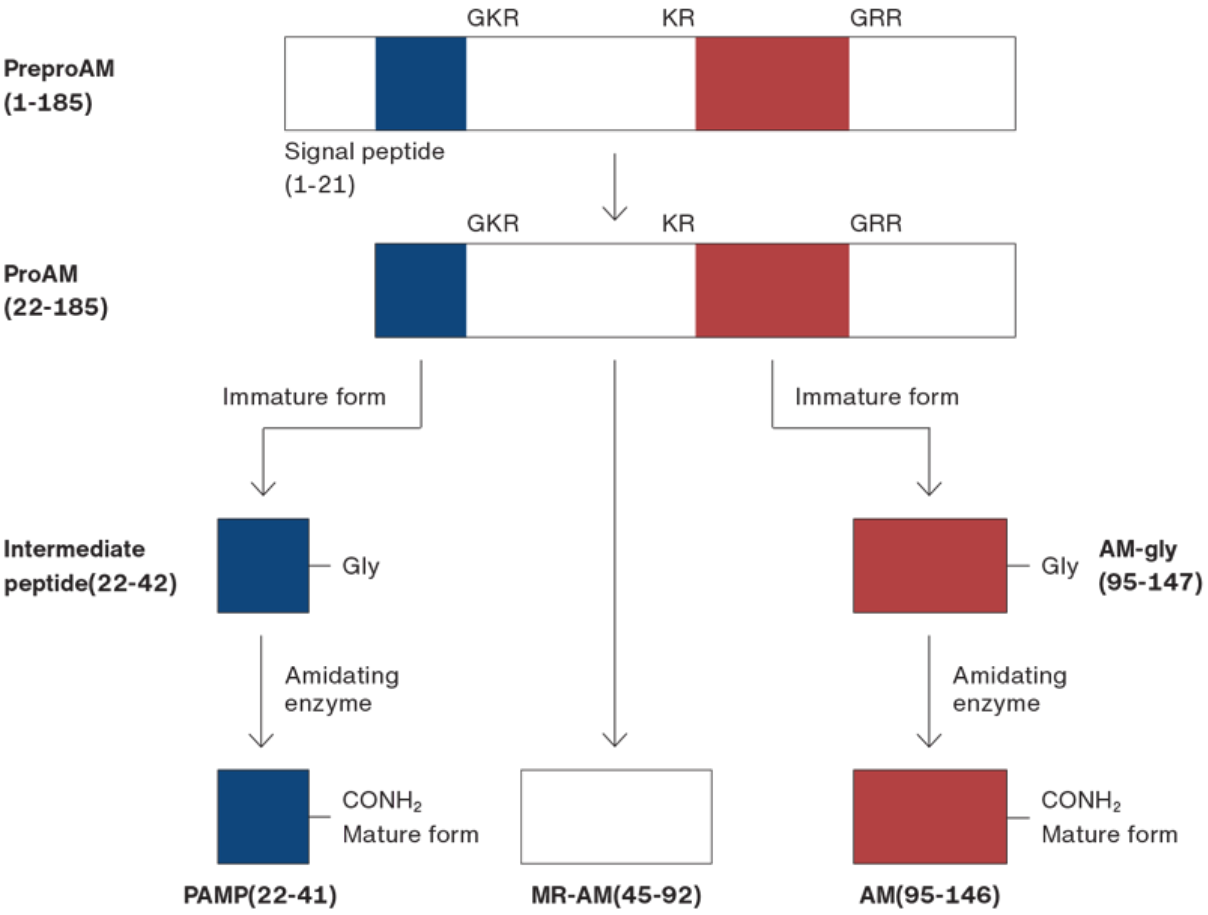
discovered in 2004 [21], also exerts pulmonary vasodilatory effects via the CGRP receptor, and that effect is mediated in part by NO release [22]. Like AM, AM-2/intermedin also reduces pulmonary artery pressure in hypoxia-induced PH [23].

Plasma adrenomedullin levels in pulmonary hypertension

Biosynthesis of adrenomedullin

AM mRNA is translated to preproAM(1-185) (**Figure 2**), after which signal peptide is removed to produce proAM(22-185) [24]. AM is produced from proAM in a two-step enzymatic reaction [25]. First, proAM is cleaved to glycine-extended AM. This is followed by enzymatic amidation, which converts AM-glycine to active mature AM containing a C-terminal amide structure [25] (Figure 2). In an early study, radioimmunoassay showed that plasma AM levels were 3.3 fmol/ml in healthy subjects [2], which was confirmed by a subsequent study [26]. Thereafter, a chemiluminescent immunoassay for mid-regional proAM (MR-proAM) was developed [27] (Figure 2). MR-proAM is inactive and stable. Consequently, plasma MR-proAM levels are about 20-30 times higher than plasma AM levels. In general, sex, age and circadian variation do not affect plasma AM levels [28]. Although expression of AM mRNA and peptide is widely distributed in various tissues, the main source of plasma AM is now thought to be the vasculature [29] as AM mRNA is more prominently expressed in vascular endothelial and smooth muscle cells than in the adrenal gland [3, 4].

FIGURE 2 Biosynthesis of adrenomedullin (AM). AM, mid-regional proAM (MR-proAM) and pro-adrenomedullin N-terminal 20 peptide (PAMP) are synthesised from the same AM precursor (preproAM: 185 amino acids). Removal of the signal peptide yields proAM, which is then processed to glycine-extended AM (AM-gly), glycine-extended PAMP and MR-proAM. AM-Gly and glycine-extended PAMP are inactive intermediate forms of AM and PAMP. AM-Gly and glycine-extended PAMP are then converted to active AM and PAMP with a C-terminal amide structure through enzymatic amidation.



AM is basically a local autocrine and paracrine factor. The active AM-mature can bind to receptors expressed in local cells where it exerts its effect. On the other hand, some of AM-glycine is secreted without converting to AM-mature. Therefore, if AM-glycine is secreted, it cannot bind to receptors expressed in local cells and is released into the circulation, where it becomes the major molecular form. In fact, the AM-mature/(AM-glycine + AM-mature) ratio in the plasma is much lower than in cardiac tissue [30].

Plasma adrenomedullin levels in pulmonary hypertension

Elevation of plasma AM concentrations in PH was first noticed in a monocrotaline-induced rat model of PH [31]. That result suggested that increased AM may contribute to a mechanism to counteract the increase in PAP. Subsequently, plasma AM levels were also found to be elevated in PH rats exposed to a hypobaric hypoxic environment [32]. In humans, the relationship between plasma AM concentrations and haemodynamics was investigated in patients with PH related to mitral stenosis [33]. Their plasma AM levels correlated with mean

pulmonary artery pressure, total pulmonary vascular resistance and pulmonary vascular resistance, and their plasma AM levels decreased significantly after percutaneous mitral commissurotomy. These results suggest that increased plasma AM levels may help attenuate the increased pulmonary arterial resistance in secondary PH related to mitral stenosis. In another study of primary and secondary severe PH, plasma AM levels were found to significantly correlate with mean right atrial pressure, stroke volume, total pulmonary resistance, mean pulmonary artery pressure and plasma atrial natriuretic peptide levels [34]. During a long-term follow-up period, plasma AM levels significantly increased in association with increases in total pulmonary resistance. Plasma AM levels have also been measured in younger patients with primary and secondary PH, which showed that AM levels are elevated even in PH patients younger than 20 years of age [35]. These results suggest that plasma AM levels increase in proportion to the severity of PH, irrespective of whether the aetiology is primary or secondary and regardless of patient age.

Plasma adrenomedullin levels and prognosis in pulmonary hypertension

Recently, plasma AM levels were measured in patients with atrial septal defect (ASD) and without PH (controls), patients with ASD with PH (ASD-PH) and patients with idiopathic/hereditary PH (I/H-PH). Compared to the controls, plasma AM levels increased in patients with ASDPAH or I/H-PH. Moreover, plasma AM levels in patients with PH increased more in non-survivors than in survivors [36]. More recently, it was reported that plasma MR-proAM levels were significantly higher in congenital heart disease patients with PH than in those without PH. In those patients, plasma MR&proAM levels correlated significantly with PAP, and MR&proAM was significantly higher in deceased patients than in survivors [37]. Plasma AM levels also correlated with haemodynamics, six-minute walk distance and NT-proBNP levels, and with the European Society of Cardiology/European Respiratory Society and Registry to Evaluate Early and Long-term PAH Disease Management risk scores. Furthermore, patients with higher AM levels had poorer survival than those with lower AM levels [36]. These results indicate that plasma AM levels are elevated in both primary and secondary PH, that they are closely related to pulmonary circulation haemodynamics and that they may be a useful prognostic indicator.

Therapeutic effects of adrenomedullin

Long-term adrenomedullin infusion in experimental pulmonary hypertension

In rats with monocrotaline-induced PH, chronic infusion of AM significantly lessened the increase in right ventricular systolic pressure, right ventricular hypertrophy and medial thickening in the pulmonary artery [38]. Similarly, in rats with PH induced by exposure to a hypobaric hypoxic environment, chronic AM infusion decreased mean pulmonary artery pressure, right ventricular hypertrophy and the relative medial thickness in pulmonary arteries [39]. These results suggest that AM ameliorates the progression of pulmonary vascular remodelling in rat both PH models.

Effect of adrenomedullin inhalation in pulmonary hypertension

Inhalation of aerosolised prostacyclin or its analogue, iloprost, has been shown to cause pulmonary vasodilation without systemic hypotension in patients with PH [40]. The acute effect of aerosolised AM on pulmonary artery pressure has been investigated in newborn piglets with PH related to surfactant depletion. It was observed that aerosolised AM reduced mean PAP without lowering systemic arterial pressure and improved arterial oxygen tension [41]. In rats with monocrotaline-induced PH, long-term repetition of AM inhalation markedly decreased mean PAP and pulmonary vascular resistance and attenuated increases in medial wall thickness in peripheral pulmonary arteries [42]. Thus, inhalation of AM appears to be a potentially powerful approach to improving the pulmonary haemodynamics and/or inhibiting the development of PH.

Adrenomedullin gene therapy and the mechanism of the beneficial effects of adrenomedullin

Transtracheal transfer of the CGRP gene into bronchial epithelial cells was shown to attenuate chronic hypoxia-induced PH in mice [43]. In another study, polyethylene glycol-based block catiomer, a nonvial gene delivery system, enabled induction of highly active luciferase gene expression in mouse lungs after intratracheal administration [44]. Using that system, a therapeutic plasmid bearing the human AM gene was intratracheally administered to rats with monocrotaline-induced PH. Three days after administration, right ventricular pressure was significantly reduced, and significant levels of human AM mRNA were detected within the lungs [44]. In addition, after a hypoxia-induced PH model was induced in mice homozygous (AM(+/+)) or heterozygous (AM(+/−)) for the AM gene, medial pulmonary arterial wall thickness and hypoxia-induced pulmonary reactive oxygen species production were both significantly greater in AM(+/−) than AM(+/+) mice [45]. Administration of exogenous AM or hydroxy-TEMPO normalised pulmonary vascular medial wall thickness in both AM(+/+) and AM(+/−) mice, suggesting that endogenous AM may act as an antioxidant. In an in vitro model of human PH, pulmonary fibroblasts and pulmonary vascular endothelial cells secreted the largest amounts of AM-2/intermedin, and their secretion was enhanced by hypertensive stretch [46]. AM-2/intermedin suppressed PDGF-mediated pulmonary vascular smooth muscle cell migration and stretch-induced proliferation of pulmonary fibroblasts, which suggests that AM-2/intermedin plays an important counter-regulatory role in PH.

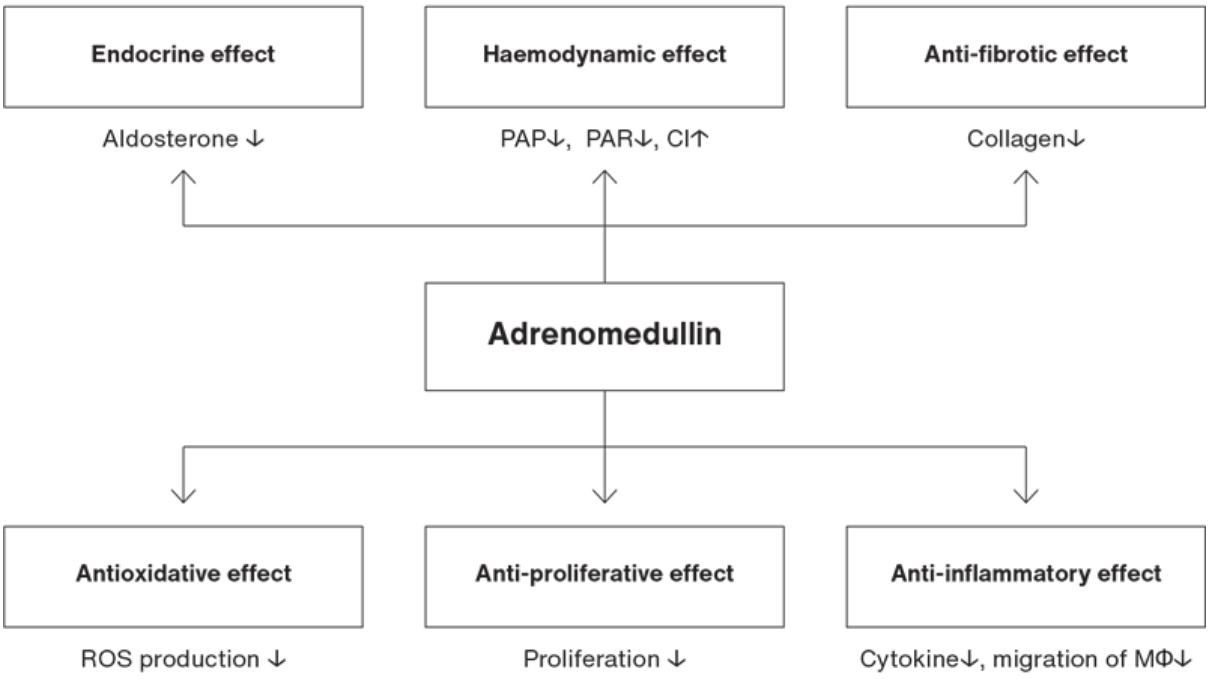
Acute therapeutic effects of adrenomedullin in human pulmonary hypertension

In patients with PH, infusion of AM produced a significant increase in the cardiac index and decreases in pulmonary vascular resistance, mean systemic arterial pressure and plasma aldosterone levels without changing PAP [47]. In contrast, inhalation of AM significantly decreased pulmonary vascular resistance and mean PAP without affecting systemic arterial pressure or heart rate. AM inhalation does not affect blood pressure reduction, potentially because of the weak increase in plasma AM concentration (AM inhalation: 12 to 23 fmol/ml: AM infusion: 15 to 48 fmol/ml). In addition, inhalation of AM significantly increased peak oxygen consumption during exercise. Thus, acute infusion and inhalation of AM may have beneficial effects on pulmonary haemodynamics in patients with PH [48].

Conclusion

Because AM receptors are abundantly expressed in the pulmonary vasculature, AM exerts a strong pulmonary vasodilatory effect. The pulmonary vasodilation induced by AM is mediated in part via nitric oxide. Plasma AM levels are increased in proportion to disease severity in patients with PH, making plasma AM a potential prognostic indicator in PH. Acute intravenous administration or inhalation of AM improves pulmonary haemodynamics in patients with PH. Moreover, chronically administered or inhaled AM and AM gene delivery exert protective effects against the development of PH. Thus, AM levels are closely related to the pathophysiology of PH and may be a promising therapeutic target for treatment of PH. A working hypothesis for the possible mechanism by which AM exerts its effects on PH is shown in **Figure 3**. Thus, AM has pleiotropic effects (Figure 3), and therapeutic applications of AM have been attempted in many conditions, including PH. Currently, large cohort studies are being conducted for inflammatory bowel disease and cerebral infarction [49-52]. For clinical application of AM, development of derivatives with a longer half-life is also currently underway to improve the short half-life of AM [53].

FIGURE 3 A working hypothesis for the mechanism underlying the therapeutic efficacy of adrenomedullin in pulmonary hypertension.



CI = cardiac index; PAP = pulmonary arterial pressure; PAR = pulmonary arteriolar resistance; ROS = reactive oxygen species.

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Accepted 24 October 2024

Published 15 November 2024

Conflicts of interest none. Disclosure forms provided by the authors are available with the article at ugeskriftet.dk/dmj

References can be found with the article at ugeskriftet.dk/dmj

Cite this as Dan Med J 2024;71(12): A07240498

doi 10.61409/A07240498

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