Review

Cardioprotective Effect of Adrenomedullin in Heart Failure

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Many neurohumoral factors participate in the pathophysiology of heart failure, and adrenomedullin (AM) may be involved in their derangement. This work reviews the accumulating evidence in support of a compensatory role of AM in heart failure, and describes the possible mechanisms of this role. It has been established that plasma AM levels are increased in patients with heart failure in proportion to the severity of the disease. Furthermore, recent studies suggest that plasma AM level is an independent prognostic indicator of heart failure. Thus, AM may be not only a biochemical marker for evaluating the severity of heart failure, but also a prognostic indicator of this syndrome. In patients with heart failure, AM production is increased not only in the plasma, but also in the heart. AM secretion from the failing human heart is also increased, but this increase is small and responds slowly to the stimulus. This phenomenon may be explained by the fact that AM is secreted *via* a constitutive pathway and that AM is an autocrine and/or a paracrine factor in the heart. An experiment using cultured myocytes suggested that cytokines and mechanical stress are important stimuli for AM production in the heart. Regarding the action of AM in the heart, recent studies have suggested that AM exerts an inotropic action both *in vitro* and *in vivo*. AM also attenuates cardiac hypertrophy in myocytes and inhibits proliferation and collagen production in cardiac fibroblasts. These results suggest that AM may be an antifibrotic, antihypertrophic, and positive inotropic factor in the failing and hypertrophied heart. Because AM has many cardiorenal actions, AM administration may be useful for the treatment of heart failure. Indeed, acute administration of AM has been shown to improve the hemodynamics, renal function, and hormonal parameters in patients with heart failure. Moreover, recent studies have shown that AM gene therapy or long-term AM infusion significantly improved cardiac hypertrophy and fibrosis, and prolonged the survival time in an animal model of hypertension and heart failure. In conclusion, these findings suggest that AM plays a compensatory role in the pathophysiology of heart failure and that administration of AM may be a new and promising approach for the treatment of patients with this syndrome.

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Introduction

Adrenomedullin (AM), a novel vasodilatory peptide, was discovered in human pheochromocytoma tissue in 1993 by monitoring the cyclic adenosine 3',5'-monophosphate (cAMP) activity in rat platelets (1). Subsequent studies revealed that AM exerts numerous other physiological actions in addition to its vasodilatory activity (2), and many of these actions are related to circulatory regulation and possibly to the pathophysiology of heart failure. The gene encoding AM has been shown to be highly expressed in the adrenal gland, heart, lung, and kidney (1). Nishikimi *et al.* found no obvious increment in the plasma AM levels in the draining veins of these organs (3), suggesting that plasma AM is unlikely to have originated from these specific organs. Sugo *et al.* reported that AM is markedly produced in vascular endothelial cells and smooth muscle cells (4, 5). Taken together, these results suggest that plasma AM may be produced in the systemic vascular bed rather than in particular organs (6). Based on the above-described physiological action of AM and the fact that immunoreactive AM has been detected in human plasma, AM is considered to be involved in the pathophysiology of heart failure. In this work, we review the accumulating evidence for a pathophysiological role of AM in heart failure, and clarify some of its possible mechanisms.

Plasma AM Levels in Heart Failure

Several reports, including ours, have shown that plasma AM levels are increased in patients with heart failure in proportion to the disease severity (7–9). In our study, we also investigated the relationships between plasma AM levels and other neurohumoral factors, such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and norepinephrine (7). We found no increase of plasma AM levels in patients with New York Heart Association (NYHA) class I disease, but plasma AM levels were slightly but significantly increased in patients with NYHA class II heart failure. Furthermore, they were further increased in patients with NYHA class III or IV disease. Plasma AM levels were positively correlated with the plasma ANP, BNP and norepinephrine levels and negatively correlated with left ventricular ejection fraction. After treatment of heart failure, the levels of plasma ANP and BNP decreased rapidly, whereas plasma AM decreased slowly (7). These results indicate that plasma AM levels are increased in proportion to the severity of the disease and that their increase may be related to the increased plasma volume and/or sympathetic nerve activity. This finding is consistent with that of a report which showed a strong correlation between plasma AM levels and pulmonary capillary wedge pressure (10). On the other hand, recent studies revealed that two molecular forms of AM circulate in human plasma, and the major circulating form is the inactive form, AM-glycine (11–13). Hirayama *et al.* reported that both mo-

lecular forms of AM are similarly increased in patients with heart failure (14). Thus, AM may respond to the pathophysiology of heart failure and may be a biochemical marker for the severity of heart failure. This hypothesis is supported by recent reports which showed that plasma AM level was an independent prognostic indicator of mild-to-moderate heart failure (15) and ischemic heart failure with established ischemic left ventricular dysfunction (16). Thus, AM may be not only an important biochemical marker for evaluating the severity of heart failure, but also a prognostic indicator of this syndrome. Accordingly, AM might come to be included in the routine clinical workup of patients with heart failure.

Cardiac Production of AM in Heart Failure

Although plasma AM levels are increased in heart failure, an immunohistochemical study has demonstrated that immunoreactivity for AM is also stronger in cardiac myocytes of the failing heart than in those of the normal heart (9), suggesting that AM production is increased in the failing heart. We also showed that the immunoreactivity and the gene expression of AM in the left ventricle were increased in rats with heart failure compared with normal rats (17). Interestingly, Yoshihara *et al.* recently reported that ventricular AM levels in the pressure- and volume-overloaded failing myocardium are well correlated with the degree of fetal cardiac gene expression (18), suggesting that the cardiac AM level is a marker of the failing heart. With respect to human heart failure, it has been reported that plasma AM levels are higher in the coronary sinus than in the aorta in patients with severe heart failure (19), suggesting that AM is produced in the failing human heart. We also investigated the changes of AM and ANP during right ventricular pacing in patients with heart failure in order to determine the secretion pattern of AM from the failing human heart (17). Plasma AM levels in the coronary sinus before pacing were slightly but significantly higher than those in the aorta, suggesting that the failing human heart actually secretes AM into the circulation. However, this increase was much smaller than that in the ANP. Moreover, pacing markedly increased plasma ANP levels in both the aorta and coronary sinus, whereas plasma AM levels did not change. These findings indicate that the regulation of AM secretion is different from that of ANP. The slow regulation of plasma AM levels may be due, in part, to the mechanism of AM secretion. Many hormones, including ANP, are stored in large amounts in secretory granules and are secreted *via* a regulated pathway. Therefore, ANP responds rapidly after pacing. In contrast, AM production is regulated at the levels of gene expression and AM is secreted *via* a constitutive pathway without intermediate storage in secretory granules (4, 5). Thus, a relatively chronic stimulus may be required to induce AM gene expression and secretion of AM.

Another possible explanation for the relatively small release of AM from the heart may be that AM acts as an au-

ocrine and/or paracrine factor in the heart. We previously reported that not only myocytes, but also cardiac fibroblasts produced and secreted similar levels of AM into medium (20). AM dose-dependently increases intracellular cAMP levels in myocytes and fibroblasts, and this increase is inhibited by an AM antagonist, calcitonin gene-related peptide (CGRP) (8–37), in both cell types. Thus, AM exerts an autocrine and/or paracrine action in the heart. We also investigated the effect of various neurohumoral factors and cytokines on the AM production and secretion in cultured myocytes and fibroblasts (21). Angiotensin II, endothelin, and phenylephrine did not increase the AM secretion in either cell type. In contrast, interleukin 1- β and tumor necrosis factor- α markedly increased the AM secretion and gene expression in these cells. Thus, increased cytokines rather than neurohumoral factors may be related to the increase of AM levels in heart failure. Moreover, Tsuruda *et al.* recently reported that mechanical stretch also increased AM secretion in cultured rat myocytes (22). We have also shown that acute aortic banding rapidly caused cardiac hypertrophy and increased AM levels in the heart *in vivo* (23). These results suggest that mechanical stress may also be an important stimulus for AM production in the heart.

Regarding the AM receptor, there has been considerable difficulty in identifying it. However, McLatchie *et al.* (24) recently demonstrated that a seven-transmembrane receptor, the calcitonin receptor-like receptor (CRLR), can function as either a CGRP or an AM receptor, depending on the coexpression of a new family of three single transmembrane receptor activity modifying proteins (RAMPs). RAMP1 and CRLR generate a CGRP receptor, while RAMP2, RAMP3, and CRLR produce an AM receptor (24, 25). A recent study reported that the mRNA levels of CRLR, RAMP2, and AM are increased in the atrium and ventricle in rats with heart failure (26). Another study showed that the expression of the AM receptor component gene is not altered in the kidney in rats with heart failure, despite the increase of AM gene expression (27). We also found that the expression levels of the AM, CRLR, RAMP2, and RAMP3 genes were increased in the hypertrophied heart induced by malignant hypertension (28). These results suggest that the AM system including ligand and receptor are all upregulated in left ventricular hypertrophy in this malignant hypertensive rat model. Thus, induction of the AM system in the hypertrophied and failing heart may modulate the pathophysiology of left ventricular hypertrophy or heart failure.

Cardiac Action of AM

Despite its evident importance, the pathophysiological role of increased AM in the failing heart is not yet fully understood. Previous studies have demonstrated that AM exerts many physiological actions in the heart (2). Intravenous administration of AM in animal models has been shown to significantly increase cardiac output (29), but it is difficult to

determine whether this is due to a direct action or a secondary effect *via* reduction of afterload. The first report of a direct action of AM in the heart was made by Perret *et al.*, who showed that AM exerts a negative inotropic action in perfused rat hearts (30). Other investigators reported that AM decreased both contractility and calcium ions in a dose-dependent manner in isolated rabbit cardiac myocytes through the nitric oxide (NO)/cyclic guanosine 3',5'-monophosphate (cGMP) pathway (31). Furthermore, Ikeda *et al.* (32) reported that the heart is a target organ of AM and that AM augments NO synthesis in the heart through a cAMP pathway under cytokine-stimulations. These findings suggest that increased AM in the heart may lead to a reduction in cardiac contractility through a NO/cGMP pathway, which has been shown to suppress myocardial contractility by decreasing cytoplasmic Ca^{2+} concentration. In contrast, AM has recently been reported to exert a direct inotropic effect *in vitro* and to increase myocardial contractility *in vivo* (33, 34). Szokodi *et al.* (33) reported that AM enhances cardiac contractility *via* cAMP-independent mechanisms including Ca^{2+} release from intracellular ryanodine- and thapsigargin-sensitive Ca^{2+} stores, activation of protein kinase C, and Ca^{2+} influx through L-type Ca^{2+} channels, suggesting that AM is an endogenous inotropic peptide in the failing heart. A recent study reported that intravenous administration of AM enhanced left ventricular myocardial contraction and improved left ventricular relaxation without increasing myocardial oxygen consumption in patients with old myocardial infarction with left ventricular dysfunction (34). The reason for the discrepancies among these studies is unknown at present, although they may be related to differences in experimental conditions, or in the age or species of the animals studied.

Regarding the effect of AM on cardiac hypertrophy, Tsuruda *et al.* (35) reported that AM significantly reduced the angiotensin II- and 10% fetal bovine serum-stimulated ^{14}C -phenylalanine incorporation into cardiac myocytes, and that this inhibitory effect of AM on the stimulated ^{14}C -phenylalanine incorporation was abolished dose-dependently by an AM receptor antagonist, CGRP(8–37). These results suggest that AM may act on cardiomyocytes as an autocrine or a paracrine anti-growth factor. AM has also been reported to have an effect on cardiac fibroblasts (36, 37). AM significantly reduced angiotensin II or endothelin-1 (ET-1)-stimulated 3H -thymidine and 3H -phenylalanine incorporation in a dose-dependent manner in cardiac fibroblasts, and these effects were attenuated by an AM receptor antagonist, CGRP(8–37). AM also had a dose-dependent stimulatory effect on cAMP accumulation in these cells, which was significantly attenuated by CGRP(8–37). We also reported that AM clearly inhibited DNA synthesis and collagen production in a dose-dependent manner under both basal and angiotensin II-stimulated conditions in cardiac fibroblasts (37). DNA and collagen synthesis by cardiac fibroblasts were suppressed by both 8-bromo cAMP and forskolin. Furthermore, a cAMP-

specific phosphodiesterase inhibitor decreased DNA and collagen syntheses in fibroblasts and enhanced the inhibitory effects of AM on these syntheses. Thus, these results suggest that AM has an important role in modulating the growth of cardiac fibroblasts in an autocrine or a paracrine manner at least in part *via* a cAMP signaling mechanism. We also assessed the effects of AM on cardiac hypertrophy *in vivo* (23). Both AM and hydralazine administration reduced the blood pressure by approximately 10% compared with that in untreated aortic banding rats, but a reduction of the left ventricular weight/body weight ratio was observed only in the AM-treated group. Left ventricular AM levels were 22% greater in the AM infusion group than in the untreated aortic banding group, and the plasma AM levels were about 5-fold greater in the AM infusion group compared with the non-treated aortic banding group. These results suggest that AM may play a pathophysiological role in the development of left ventricular hypertrophy induced by pressure overload. Thus, it is possible that AM may act as an antifibrotic, anti-hypertrophic, and positive inotropic factor (or possibly as a negative inotropic factor under some conditions) in the failing or hypertrophied heart.

Acute Administration of AM in Heart Failure

Although the exact pathophysiological role of increased AM in heart failure remains unknown, many physiological actions of AM, including a vasodilatory effect, diuretic and natriuretic effects, and an inhibitory effect of aldosterone secretion, *etc.*, suggest that AM is increased in heart failure as a compensatory mechanism. Administration of AM may therefore be useful for the treatment of heart failure.

Rademaker *et al.* (38) reported that AM increased plasma cAMP levels in association with dose-dependent falls in calculated peripheral resistance, mean arterial pressure, and left atrial pressure, and dose-dependent increases in cardiac output in sheep with pacing-induced heart failure. They also showed that AM increased urinary sodium excretion, creatinine and cAMP excretion, and creatinine clearance with a reduction of plasma aldosterone levels (38). We also examine the cardiovascular and renal effects of low- and high-dose intravenous infusion of AM in a rat model of heart failure (39). Low-dose AM increased urine flow and urinary sodium excretion without changes in any hemodynamic variables. In contrast, high-dose AM slightly decreased mean arterial pressure and significantly increased cardiac output in heart failure and normal rats. Infusion of high-dose AM also resulted in significant decreases in right ventricular systolic pressure and right atrial pressure only in heart failure rats. High-dose AM significantly increased glomerular filtration rate and renal plasma flow as well as urine flow and urinary sodium excretion (39). These studies imply an important pathophysiological role for AM in the regulation of pressure and volume in heart failure, and raise the possibility of a new therapeutic approach to this disease.

For this reason, we examined the effects of intravenous administration of AM on hemodynamic, renal, and hormonal responses in patients with heart failure (40). AM markedly increased the cardiac index while decreasing pulmonary capillary wedge pressure in heart failure and normal subjects. AM significantly decreased mean pulmonary arterial pressure only in patients with heart failure, and AM increased urine volume and urinary sodium excretion in both groups. Only in patients with heart failure, plasma aldosterone was significantly decreased during and after AM infusion. These results indicate that intravenous infusion of AM has beneficial hemodynamic, renal and hormonal effects in patients with heart failure. Lainchbury *et al.* also reported that AM and BNP, at plasma concentrations within the pathophysiological range, have beneficial hemodynamic, renal, and hormonal effects in patients with heart failure (41). The same group recently reported that coadministration of AM and an endopeptidase inhibitor had a beneficial effect in sheep with heart failure, and attributed this finding to the fact that AM is metabolized in part by neutral endopeptidase. In their study, coadministration of AM and endopeptidase inhibitor produced hemodynamic effects greater than those achieved during AM administration alone. Despite the larger falls in blood pressure, renal function was improved and elevations in plasma AM and cAMP were greater than those during AM administration alone (42). These results suggest that cotreatment with AM and an endopeptidase inhibitor has beneficial hemodynamic and renal effects in heart failure. Thus, combination therapy with AM and an endopeptidase inhibitor may be one possible approach for the treatment of heart failure.

Chronic Administration of AM in Heart Failure

Several reports have investigated the effect of chronic administration of AM in cardiovascular disease. Khan *et al.* (43) reported that chronically infused AM has a hypotensive effect in both normotensive rats and spontaneously hypertensive rats without an increase in urinary volume or sodium excretion at a plasma AM concentration within the physiological limit. The same group also demonstrated that chronically infused AM had a hypotensive effect accompanied by significant reductions of plasma renin activity and plasma aldosterone concentration in renovascular hypertensive and sham-operated rats at a plasma AM concentration within the physiological range (44). These results imply that chronic AM infusion has beneficial effects in hypertension and its organ damage in part *via* inhibition of the renin-angiotensin system. Yoshihara *et al.* (45) reported the beneficial effect of long-term AM infusion in pulmonary hypertension and right ventricular hypertrophy induced by the administration of monocrotaline. Chronic infusion of AM significantly lessened the increase in right ventricular systolic pressure and the ratio of right ventricular weight. AM also attenuated the medial thickening of the pulmonary artery. These results

suggest that chronic infusion of AM attenuates the pulmonary hypertension and right ventricular hypertrophy in rats treated with monocrotaline at least in part *via* an inhibitory effect of AM on the pulmonary arterial remodeling.

Furthermore, recent advances in gene therapy have made it possible to chronically elevate plasma and tissue AM levels. Dobrzynski *et al.* (46) explored the potential protective role of AM in deoxycorticosterone acetate-salt hypertension by somatic gene delivery using an adenovirus containing the human AM cDNA under the control of the cytomegalovirus promoter/enhancer. A single injection of the human AM gene resulted in a prolonged reduction of blood pressure. Human AM gene delivery improved renal function and histologically examined conditions such as glomerular sclerosis, tubular injury, luminal protein cast accumulation, and interstitial fibrosis. Human AM gene delivery caused significant decreases in left ventricular weight and cardiomyocyte diameter, which were accompanied by reduced interstitial fibrosis and extracellular matrix formation within the heart. The same investigators also reported the beneficial effects of human AM gene therapy in other hypertensive rat models (47). AM gene delivery significantly reduced blood pressure, left ventricular mass and urinary protein, and increased cAMP levels, glomerular filtration rate and renal blood flow in Dahl salt-sensitive rats. AM gene transfer reduced cardiomyocyte diameter and interstitial fibrosis in the heart. These findings showed that AM gene delivery attenuates hypertension, protects against cardiac remodeling and renal damage in hypertension, and may have significance in therapeutic applications in cardiovascular and renal diseases. We also recently reported that chronic human AM infusion exerted renoprotective effects in a rat model of malignant hypertension (48, 49). Human AM infusion significantly reduced plasma renin concentration, plasma aldosterone levels and plasma endogenous rat AM levels with a slight increase of plasma human AM levels. AM decreased urinary protein excretion, improved glomerular sclerosis, and significantly decreased the intrarenal angiotensin II levels and gene expression of transforming growth factor- β and angiotensin converting enzyme. These results suggest that increased endogenous AM plays a compensatory role in chronic hypertensive renal failure and that long-term AM infusion has renoprotective effects in this type of hypertension model, partly *via* inhibition of the circulating and renal renin-angiotensin system.

We also analyzed the effect of chronic AM infusion in a rat model of heart failure (50). Chronic human AM infusion decreased left ventricular end-diastolic pressure, right ventricular systolic pressure, right atrial pressure, and left ventricular weight/body weight without significant change in mean arterial pressure. Administration of human AM significantly decreased endogenous rat plasma AM levels. In addition, chronic AM infusion significantly decreased plasma renin concentration, aldosterone and atrial natriuretic peptide levels. Furthermore, AM infusion significantly decreased

myocardial tissue angiotensin II, ANP, and BNP levels and gene expression of ANP and BNP. Kaplan-Meier survival analysis showed that AM infusion significantly prolonged survival time. These results suggest that endogenous AM plays a compensatory role in heart failure and that long-term AM infusion may be useful for treating this syndrome.

In conclusion, current evidence suggests that AM plays an important role in fluid and electrolyte homeostasis and cardiorenal regulation in heart failure. Thus, acute administration of AM may be a new and promising approach for treatment of patients with this disease. Further investigations will be needed to examine the effects of long-term AM treatment in human heart failure.

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