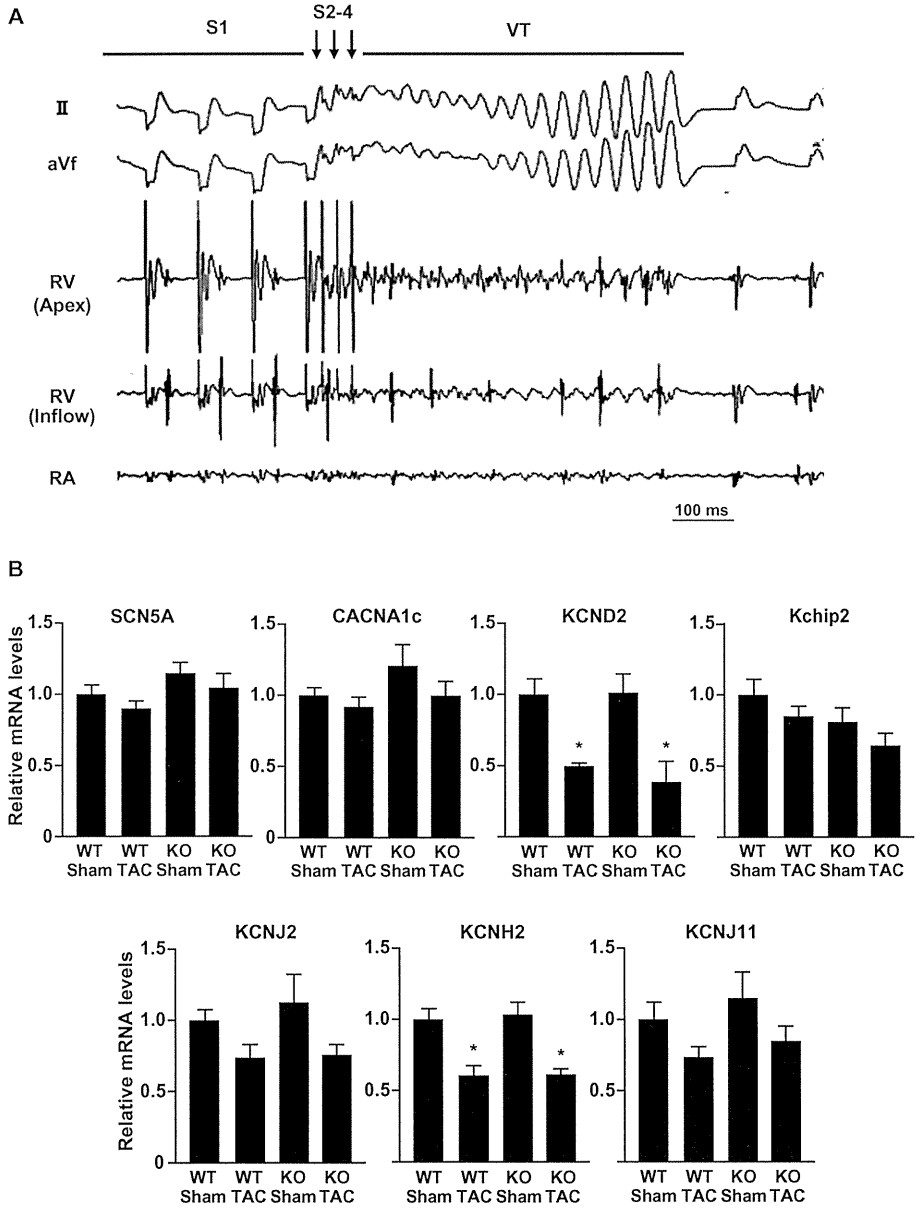

Figure 4

Induction of ventricular arrhythmias in mice with chronic pressure overload. (A) Representative traces showing polymorphic ventricular tachycardia (VT) induced by programmed ventricular stimulation in a mouse subjected to transverse aortic constriction (TAC). RV, right ventricle; RA, right atrium. Ten pacing stimuli (S1; a coupling interval is 80–90 ms) followed by 2–3 extra stimulations (S2-4) were used to induce VT. (B) Effects of chronic pressure overload on left ventricular ion channel gene transcription 4 weeks after TAC or sham operation. Graphs show relative expression levels of SCN5A, CACNA1c, KCND2, Kchip2, KCNJ2, KCNH2 and KCNJ11 mRNA normalized to corresponding GAPDH mRNA levels. The mean relative level of each mRNA in sham-operated WT mice was assigned a value of 1.0. Values are means ± SEM (n = 8 each). *P < 0.01 versus sham-operated mice in each genotype.

activates kinases that modulate Cx43 phosphorylation (Sadoshima and Izumo, 1996; Zou *et al.*, 1998; Lampe and Lau, 2004; Sovari *et al.*, 2011). Particularly, it has been recently reported that angiotensin II induces c-src TK-mediated remodelling of Cx43, which leads to the increase in sudden arrhythmic death (Sovari *et al.*, 2011). We therefore examined the tyrosine phosphorylation status and protein amount of Cx43 in TAC- and sham-operated AT1aR-KO and WT mice. We observed prominent tyrosine phosphorylation of Cx43 and a substantial reduction in tissue Cx43 protein levels in WT mice subjected to TAC (Figure 5A and B). Both the TAC-induced increase in tyrosine phosphorylation of Cx43 (Figure 5A and B) and the reduction in the levels of Cx43 protein were diminished in AT1aR-KO mice (Figure 5A and C). AT1aR-mediated signalling thus appears to play a key role in a pressure overload-induced increase in the tyrosine phosphorylation of Cx43 that leads to diminished tissue levels of Cx43 protein (Toyofuku *et al.*,

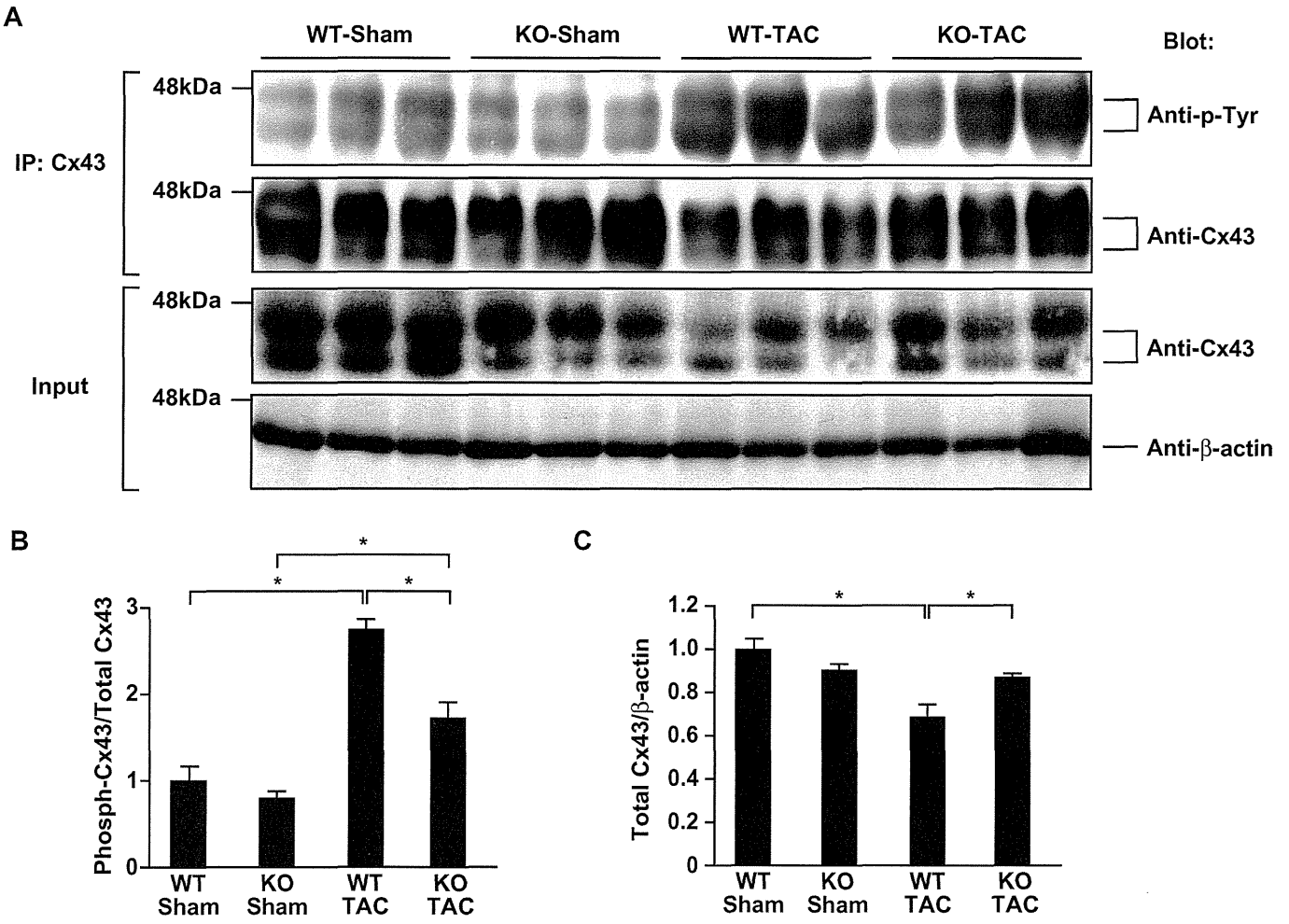

Figure 5

Phosphorylation status and tissue level of Cx43 protein in the left ventricles of angiotensin II type 1a receptor knockout (AT1aR-KO) and wild-type (WT) mice 4 weeks after transverse aortic constriction (TAC) or sham operation. (A) Protein obtained from left ventricles was immunoprecipitated (IP) with anti-Cx43 antibody. The panels show the immunocomplexes separated by electrophoresis and blotted with the indicated antibodies (Blot). Crude lysates were analysed by Western blotting to control for variation in protein expression (Input). (B) The ratios of phospho-Cx43 to total Cx43 in immunoprecipitation assays evaluated by quantitative densitometry. The mean value of the phospho-Cx43/total Cx43 ratios in sham-operated WT mice was assigned a value of 1.0. Values are means ± SEM ($n = 3$ each). *$P < 0.05$. (C) The results of quantitative densitometric analysis of left ventricular Cx43. The mean relative levels (corrected by β-actin level) of total Cx43 in sham-operated WT mice was assigned a value of 1.0. Values are means ± SEM ($n = 3$ each). *$P < 0.05$.

2001). It may be that the preservation of Cx43 underlies the reduced arrhythmogenicity of chronic pressure overload in AT1aR-KO mice.

Acute blockade of AT1R signalling decreased induced ventricular tachyarrhythmias in mice with chronic pressure overload

Finally, we tested whether acute pharmacological blockade of AT1R signalling would decrease tachyarrhythmias induced in mice with chronic pressure overload. We compared the effects of vehicle to those of 0.3 mg·kg^{-1} EXP-3174, an active metabolite of the AT1R blocker losartan, which reduced systolic BP as much as 10.2 mmHg in WT mice (Figure 6A). Intravenous administration of EXP-3174 or vehicle to mice after 4 weeks of TAC did not significantly affect heart rates 60 min after administration under anaesthesia (Figure 6B). In addition, cardiac hypertrophy evaluated based on heart weight-to-body weight ratios was similar in the two groups (Figure 6C and Table 2). Meanwhile, the induction rate of VT in the mice acutely administered EXP-3174 was significantly lower than in the mice administered vehicle (Figure 6D and Table 2). The CX43 protein level was decreased in mice subjected to TAC with vehicle treatment (Figure 6E, left lane), compared with sham-operated mice with vehicle treatment (Figure 6, right lane). Acute EXP-3174 treatment restored the decreased CX43 protein level in mice subjected to TAC (Figure 6E, middle lane). The relative amount of Tyr^{265}-phosphorylated Cx43 to total Cx43 was tended to be increased in mice subjected to TAC with vehicle treatment

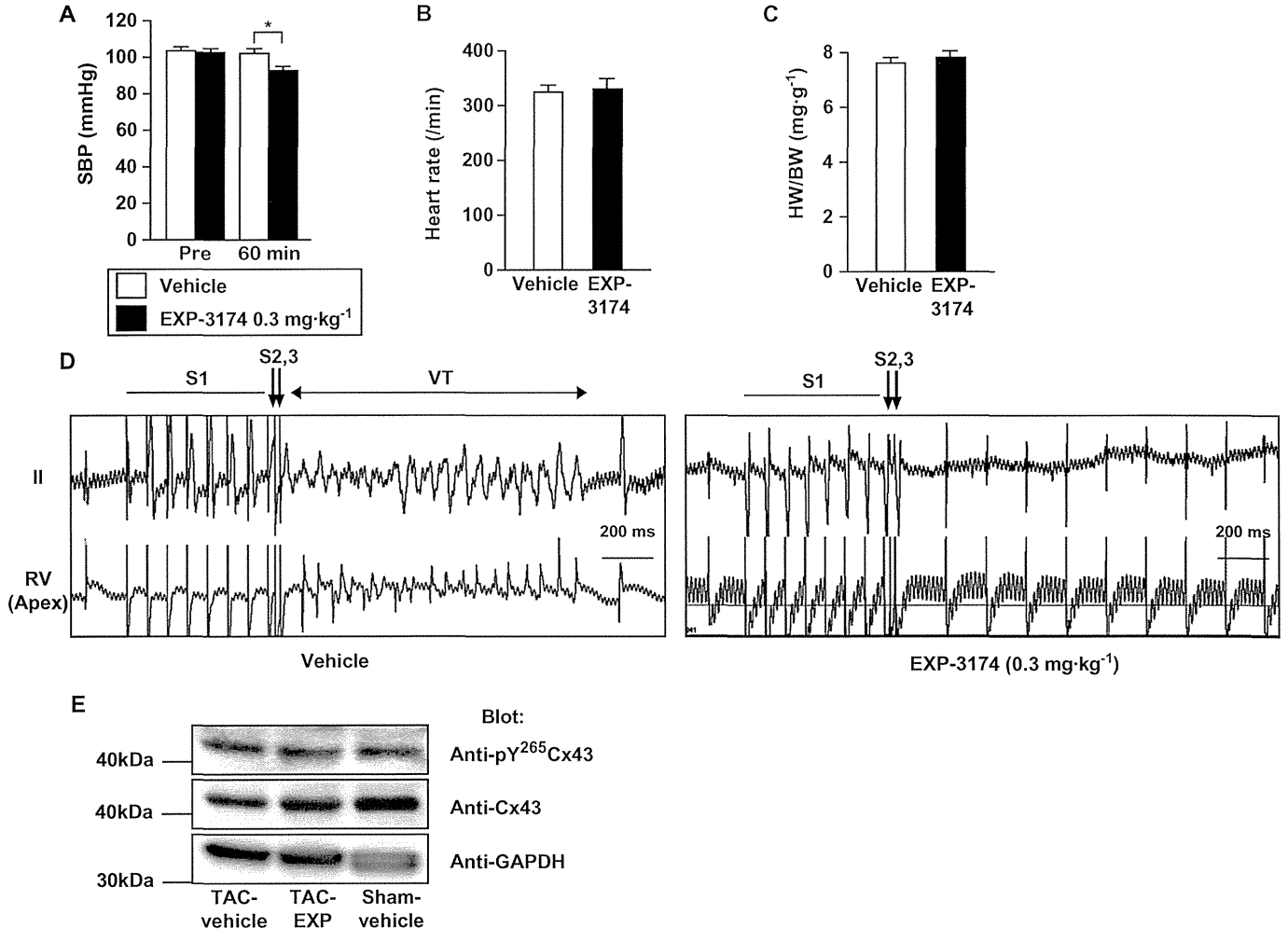

Figure 6

Effects of acute pharmacological blockade of AT1R signalling on the inducibility of ventricular tachycardia (VT) in mice with chronic pressure overload. (A) Systolic BP before (Pre) or 60 min after injection of control wild-type (WT) mice with vehicle or 0.3 mg·kg^{-1} of EXP-1374 ($n = 6$ each). *$P < 0.05$. (B) Heart rates recorded after 4 weeks of transverse aortic constriction (TAC) in mice treated with vehicle ($n = 11$) or EXP-3174 ($n = 12$) under anaesthesia. (C) Heart weight-to-body weight (HW/BW) ratios after 4 weeks of TAC in mice treated with vehicle ($n = 11$) or EXP-3174 ($n = 12$) treatment. (D) Representative traces recorded during programmed ventricular stimulation in a mouse subjected to 4 weeks of TAC and then acutely injected with vehicle (left panel) or EXP-3174 (right panel). RV, right ventricle; VT, ventricular tachyarrhythmias. Ten pacing stimuli (S1; a coupling interval is 80 ms) followed by 2 extra stimulations (S2, S3) were used to induce VT. (E) Representative Western blots showing Tyr^{265}-phosphorylated Cx43, total Cx43 and GAPDH protein in a mouse subjected to 4 weeks of TAC or sham-operation and then acutely injected with vehicle (Sham-vehicle and TAC-vehicle) or EXP-3174 (TAC-EXP). Protein obtained from left ventricles was separated by electrophoresis and blotted with the indicated antibodies (Blot). Two different experiments gave essentially identical results.

(Figure 6E, left lane) compared with that in sham-operated mice (Figure 6E, right lane) and that in mice subjected to TAC with EXP-3174 treatment (Figure 6E, middle lane). The result is consistent with the results obtained in the experiments using AT1aR-KO mice and suggests that acute EXP-3174 treatment affected tyrosine phosphorylation of Cx43 and then restored Cx43 protein levels, thereby reducing the increased arrhythmogenicity in mice subjected to TAC. These results suggest that acute inhibition of AT1R-mediated signalling can provide a significant protective effect against induction of VT in hypertrophied hearts, and further supports our hypothesis that AT1R-mediated signalling directly modulates arrhythmogenicity in hypertrophied ventricles.

Discussion and conclusions

The evidence suggests that angiotensin II signalling contributes to adverse electrical remodelling in patients with heart failure. It has been shown that angiotensin II signalling plays an important role in the structural remodelling of the heart (e.g. cardiomyocyte hypertrophy and cardiac fibrosis), which can contribute to the increase in arrhythmogenicity, during the development and progression of cardiomyopathy. However, it remained unclear whether this signalling makes a direct contribution to the altered electrical properties that increase cardiac arrhythmogenicity independently of structural remodelling. In the present study, we compared the

susceptibility to arrhythmia of WT and AT1aR-deficient mice following induction of cardiac hypertrophy through chronic pressure overload. We found that AT1aR-KO mice with TAC developed cardiac hypertrophy in exactly the same manner as WT mice with TAC, as was seen previously in a study of AT1aR-KO mice subjected to abdominal aortic banding (Harada *et al.*, 1998b). Nevertheless, induction of VT by programmed stimulation was significantly diminished in AT1aR-KO mice, as compared to WT mice. The overall ventricular levels of several ion channel genes, including those responsible for the I_{Na}, $I_{Ca\text{-}L}$, I_{TO}, I_{K1}, I_{Kr} and $I_{K\text{-}ATP}$, were comparable in the two genotypes, although the protein levels of these ion channels and their spatial distribution throughout the ventricles were not assessed in detail. We found that there was significantly less tyrosine phosphorylation of Cx43 in AT1aR-KO than WT mice subjected to TAC, and that levels of Cx43 protein were better preserved in AT1aR-KO mice, which would be expected to ameliorate the functional deterioration of junctional conductance caused by loss of Cx43. Collectively, these results demonstrate that AT1aR-mediated signalling makes a direct contribution to the increase in arrhythmogenicity in hypertrophied hearts independently of structural remodelling, in addition to its potential effects on the increased susceptibility to arrhythmias by promoting structural remodelling.

Harada *et al.* (1998a) previously showed that the incidence of arrhythmias is lower in AT1aR-KO than WT mice following ischaemia-reperfusion. Although the mechanism involved in reperfusion-induced arrhythmias, which occur in an acute setting, may differ from the one underlying the arrhythmogenicity induced by chronic pressure overload, there is the possibility that a common molecular mechanism underlies the anti-arrhythmogenic effects of AT1aR inhibition under both acute and chronic pathological conditions. In this regard, the observed reduction in tyrosine phosphorylation of ventricular Cx43 in AT1aR-KO mice is intriguing. That acute blockade of AT1R signalling by EXP-3174, an active metabolite of AT1R blocker losartan, significantly reduced the induction rate of VT in mice with chronic pressure overload supports this notion. Lynch *et al.* (1999) also reported the acute anti-arrhythmic effect of EXP-3174 in canine model of acute myocardial ischaemia. In this report, however, the authors could not preclude the possible existence of AT1R-independent mechanisms underlying the anti-arrhythmic effect of EXP-3174 (Lynch *et al.*, 1999). Further studies are necessary to assess the possible contribution of AT1R-independent mechanism to the anti-arrhythmic effect of EXP-3174 observed in this study. In addition, EXP-3174 reduced systolic BP as much as 10.2 mmHg in WT mice (Figure 6A). There is another possibility that acute reduction of systemic BP may influence the effect of EXP-3174 on mice subjected to TAC.

Phosphorylation of Cx43 affects the function of gap junctions (Warn-Cramer and Lau, 2004) largely by facilitating the degradation of Cx43, which leads to functional deterioration of the junction (Saffitz *et al.*, 1999; Toyofuku *et al.*, 2001; Warn-Cramer and Lau, 2004). Such gap junctional dysfunction is thought to be involved in the increased arrhythmogenicity seen in models of chronic cardiac hypertrophy and heart failure (Danik *et al.*, 2004; Poelzing and Rosenbaum, 2004; van Rijen *et al.*, 2004). Altered Cx43 phosphorylation is also reportedly involved in the increased arrhythmogenicity seen after ischaemia-reperfusion, although the precise molecular mechanisms linking Cx43 phosphorylation and arrhythmogenicity appear to differ in acute and chronic disease models. Nonetheless, Cx43 may be the common target of AT1aR-mediated signalling leading to increased arrhythmogenicity in both acute and chronic pathological conditions. Consistent with that idea, cardiac overexpression of ACE in mice was shown to reduce Cx43 expression via c-src TK-mediated pathways and increase susceptibility to cardiac arrhythmias and sudden death in the absence of structural remodelling (Xiao *et al.*, 2004; Kasi *et al.*, 2007; Sovari *et al.*, 2011). In addition, transgenic rats overexpressing human renin and angiotensinogen also died from lethal arrhythmias, and Cx43 disorganization was detected in the ventricles of those rats (Fischer *et al.*, 2007). All of these findings implicate angiotensin II signalling in post-translational modification of Cx43 that increases the arrhythmogenicity of diseased hearts.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Adrenomedullin in Cardiovascular Disease: A Useful Biomarker, its Pathological Roles and Therapeutic Application

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Abstract: Many neurohumoral factors play important roles in the regulation of the cardiovascular system and in the pathophysiology of cardiovascular disease. Adrenomedullin (AM) is a potent vasodilatory peptide originally discovered in the acid extract of human pheochromocytoma tissue but now known to exert a variety of effects within the cardiovascular system. AM expression is widely distributed throughout the cardiovascular system and has been identified in the heart, lungs, blood vessels and kidneys. In addition, the co-localization of AM and its receptor components suggest AM acts as an autocrine and/or paracrine factor to play a key role in the regulation of cardiovascular function. Evidence also strongly suggests that cardiovascular disease is associated with elevated levels of AM in plasma and tissue. In this review, we describe the pathophysiological changes in plasma and local AM associated with myocardial infarction, heart failure and pulmonary hypertension. We also describe the clinical application of AM in cardiovascular disease from the viewpoints of diagnosis and treatment.

Keywords: Adrenomedullin, myocardial infarction, heart failure, pulmonary hypertension, prognosis, ischemia/reperfusion.

INTRODUCTION

Adrenomedullin (AM) was discovered in 1993 in the acid extract of human pheochromocytoma tissue by monitoring the cAMP activity evoked by the extract in rat platelets [1]. Since then, strong expression of AM mRNA has been observed in the cardiovascular system and related organs, including the adrenal gland, heart, lung, kidney and blood vessels [2] and subsequent studies demonstrated that AM has a variety of biological actions related to the regulation of cardiovascular function [3]. For that reason, AM is considered to be a cardiovascular peptide. In addition, tissue and plasma AM levels are increased in various cardiovascular diseases, suggesting the potential involvement of AM in the pathophysiology of cardiovascular disease. Here we describe recent advances in our understanding the role of AM in the pathophysiology of acute myocardial infarction, heart failure and pulmonary hypertension. We also discuss the clinical application of AM for both the diagnosis and treatment of cardiovascular disease.

1. ADRENOMEDULLIN (AM) IN ACUTE MYOCARDIAL INFARCTION

1.1. Plasma AM Levels in Acute Myocardial Infarction

Early studies showed that plasma AM levels increase immediately after the onset of acute myocardial infarction and then decline gradually, and that patients with congestive heart failure have higher plasma AM levels than those without congestive heart failure [4]. When, in a subsequent study, plasma AM levels were serially measured in patients with acute myocardial infarction over a 4-week period, it was found that plasma AM reaches a peak 24 to 48 h after the onset of symptoms and then remains higher than in healthy subjects at all sampling points for at least a week (Fig. **1**) [5]. This study also showed that plasma AM levels were higher on admission in patients with heart failure than in those without heart failure, and that the levels correlated positively with peak creatine phosphokinase and left ventricular end diastolic volume index, and correlated negatively with left ventricular ejection fraction [2]. The increase in plasma AM seen during the acute phase of myocardial infarction was thus in proportion to the clinical severity. In another study, which examined plasma AM levels before and immediately after reperfusion using percutaneous transluminal coronary angioplasty, it was found that the intervention did not rapidly affect the plasma AM level [6].

AM is produced from AM precursor through a two-step enzymatic reaction. First the signal peptide[1-21] is removed from preproAM[1-185], yielding proAM[22-185] [7]. Then proAM is cleaved by the processing enzyme into three products: glycine-extended AM (AM-Gly), glycine-extended PAMP and mid-regional proAM (MR-proAM) (Fig. **2**). AM-Gly is a 53-amino acid, inactive intermediate that is converted through enzymatic amidation to active mature AM (AM-m), a 52-amino acid peptide containing a C-terminal amide (Fig. **2**). Asakawa *et al.* investigated the pathophysiological significance of the two molecular forms of AM in

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the plasma and urine of patients with acute myocardial infarction [8]. They found that plasma AM-m and AM-Gly (Fig. **2**) were both elevated on admission in patients with acute myocardial infarction and reached a peak 24 h after the onset of symptoms. In addition, plasma AM-m and AM-Gly levels were significantly correlated with those of BNP and with pulmonary arterial pressure. Urinary excretion of AM-m and AM-Gly was also elevated on admission, reached a peak 12 h after the onset of symptoms, and was significantly correlated with urinary sodium excretion. AM-m levels were significantly correlated with AM-Gly in both the urine and plasma; however, there was no significant correlation between plasma and urinary AM levels. These results suggest that levels of both molecular forms of AM are elevated in the urine and plasma during acute myocardial infarction, and that urinary AM and plasma AM are regulated by different mechanisms. The higher concentrations of AM in plasma and urine during acute myocardial infarction may exert a protective effect against further increases of peripheral and pulmonary vascular resistance and oliguria.

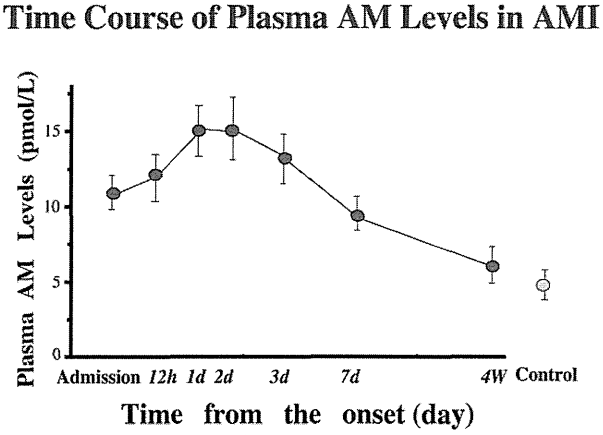

Fig. (1). Time course of plasma AM levels in patients with acute myocardial infarction (AMI). Plasma AM levels were increased at the time of admission. The AM levels thereafter increased further and reached a peak approximately 24 ~ 48 hours after the onset of acute myocardial infarction. Plasma AM levels remained raised until seven days and then gradually declined.

To determine whether cardiac synthesis of the mature forms of AM is accelerated in patients with acute myocardial infarction, Yasu *et al.* used a specific immunoradiometric assay to measure AM-m and AM-Gly, in the aorta and coronary sinus (CS) [9]. Plasma levels of both AM-m and AM-Gly in the aorta and CS were higher in patients with acute myocardial infarction than in controls. In addition, CS-aortic step-up of AM-m, an index of myocardial production of AM-m, was significantly greater in acute myocardial infarction patients than in controls, though there was no significant change in the CS-aortic step-up of AM-Gly. Patients with left ventricular dysfunction had a significantly greater CS-aortic AM-m step-up than those without left ventricular dysfunction, and AM-m in the aorta and CS correlated negatively with the left ventricular ejection fraction. From these results, it was concluded that myocardial synthesis of AM-m is accelerated in patients with acute myocardial infarction, especially patients with critical left ventricular dysfunction. It was hypothesized that increased myocardial synthesis of active AM may protect against cardiac dysfunction, myocardial remodeling, or both after the onset of acute myocardial infarction.

1.2. Plasma AM and MR-proAM as Predictors of Acute Myocardial Infarction

Plasma AM levels may be a useful as a predictor of survival after acute myocardial infarction. Nagaya *et al.* [10]. measured plasma AM and other clinical and hemodynamic variables on day 2 after myocardial infarction in 113 patients and then followed up for 25 months. Univariate Cox proportional hazards analysis showed that plasma AM, age, coronary reperfusion, maximum creatine kinase concentrations, pulmonary congestion, pulmonary capillary wedge pressure, cardiac index and left ventricular ejection fraction were all significantly related to mortality. Multivariate Cox proportional hazards analysis showed that among the non-invasive variables, only plasma AM was an independent predictor of mortality after myocardial infarction. The Kaplan-Meier survival curves based on the median plasma AM concentration showed that patients with higher plasma AM had a higher mortality than those with lower plasma AM, suggesting plasma AM can be used as a prognostic indicator in acute myocardial infarction. Richard *et al.* [11] compared the ability of several neurohumoral factors to serve as prognostic markers after acute myocardial infarction. They showed that plasma AM levels have a significant inverse relation with left ventricular function, which is comparable to that of norepinephrine. They also showed that plasma AM is predictive of death in the 2 years after myocardial infarction, but this relation was generally weaker than that observed for N-terminal proBNP (NT-proBNP). It was speculated that the prognostic value of AM was weakened by the fact that its elevation in plasma is mediated by a variety of mechanisms. Hence AM appears to be an indirect reflector of left ventricular function with a weaker association with left ventricular size, contractile function and prognosis than BNP or NT-proBNP.

MR-proAM, another part of the AM precursor, has been identified in plasma (Fig. **2**), and an MR-proAM assay has been developed [12].This peptide is inactive and stable and has a longer half-life in plasma than AM, because it does not bind to a receptor (Table **1**). On a molar basis, plasma MR-proAM levels are about 20-30 times higher than AM levels [12]. Khan *et al.* [13] first investigated the cardiovascular prognostic value of MR-proAM in 983 patients with acute myocardial infarction. They found that MR-proAM was higher in patients who had died or had survived with heart failure than in other survivors. Using a multivariate binary logistic model, log MR-proAM (odds ratio 4.22) and log NT-proBNP (odds ratio 3.20) were found to be significant independent predictors of death or heart failure. The areas under the receiver-operating characteristic (ROC) curve for MR-proAM and NT-proBNP were similar, so that MR-proAM provided further risk stratification in patients with NT-proBNP levels above the median. Thus MR-proAM appears to be a powerful predictor of adverse outcome, especially in those with elevated NT-proBNP. MR-proAM may also be a clinically useful marker of prognosis after acute myocardial infarction. The same group assessed MR-proAM levels on admission and discharge in 745 myocardial

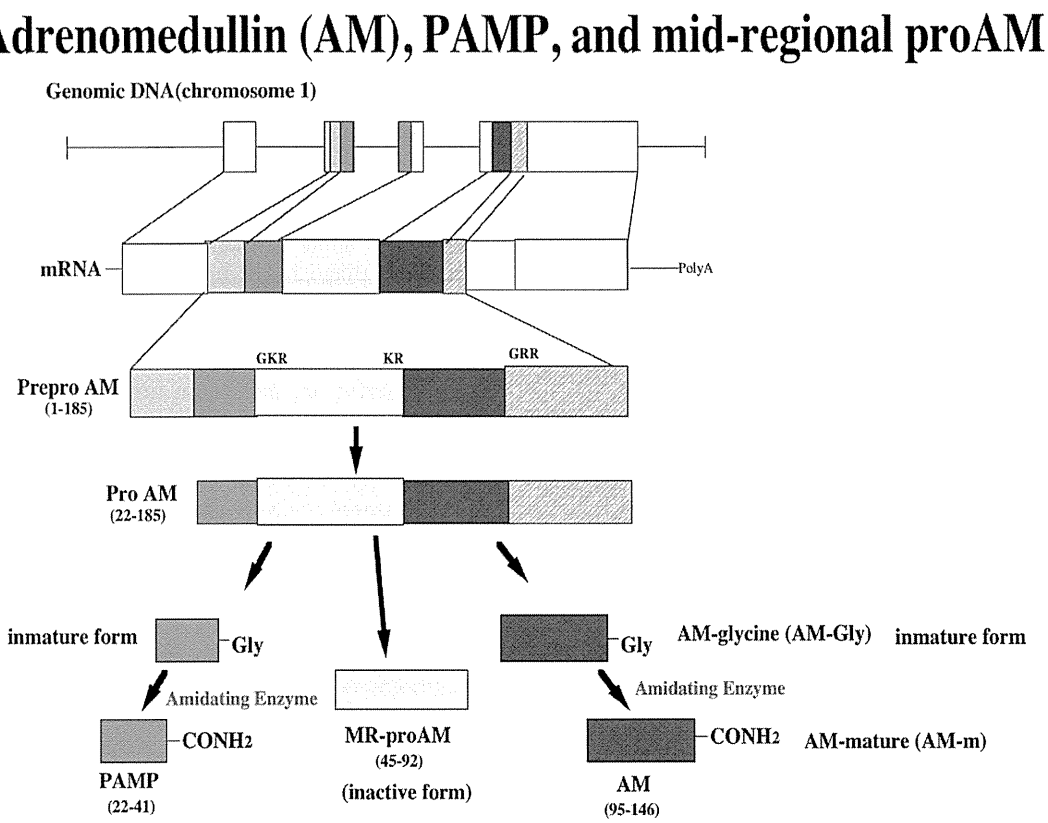

Fig. (2). Schematic diagram of the AM gene, AM mRNA, preproAM, proAM and the biosynthesis of AM, proadrenomedullin N-terminal 20 peptide (PAMP) and mid-regional proAM (MR-proAM).

infarction patients without ST-elevation [14]. Plasma MR-proAM was elevated on both admission and discharge, and the levels on admission were particularly associated with early (< 30 days) mortality. Multivariate adjusted Cox regression models revealed that plasma MR-proAM levels on both admission and discharge were associated with mortality and heart failure. From these results, they concluded that plasma MR-proAM levels are prognostic for death and heart failure in cases of myocardial infarction with or without ST-elevation [14]

Recently, Klip *et al.* [15] assessed the cardiovascular prognostic value of MR-proAM and compared it to BNP and NT-proBNP with respect to death or a composite end point in a 214-patient subset from the OPTIMAAL study who had developed heart failure after an acute myocardial infarction. In multivariable Cox proportional hazard models, a doubling of MR-proAM led to a 3.02 times increase in the risk of mortality and a 1.77 times increase in the risk of reaching the composite end point. ROC curves indicated that MR-proAM was a stronger predictor of mortality than BNP or NT-proBNP. Moreover, MR-proAM significantly enhanced risk classification and improved integrated discrimination, as compared to BNP and NT-proBNP. Thus MR-proAM appears to be a promising biomarker with greater prognostic value for mortality and morbidity in patients with heart failure after an acute myocardial infarction than are BNP and NT-proBNP. The discrepancy between these findings and those of an earlier study may be due to differences in disease severity and/or difference between AM and MR-proAM [15].

Table 1. Comparison of AM with MR-proAM

	AM	MR-proAM
Amino acid length	52, proAM[95-146]	48, proAM[45-92]
Molecular weight	6028	5147
Hormonal activity	(+)	(-)
Clearance in the lung,	(+)	(-)
Excretion from the kidneys	(+)	(+)
Half life	22 min	unknown, but longer than AM
Plasma levels in normal subjects	3-10 fmol/ml	300 fmol/ml

1.3. Role of Cardiac AM in the Pathophysiology of Acute Myocardial Infarction

As described above, plasma AM is elevated early after acute myocardial infarction. Nagaya *et al.* first showed that expression of both AM peptide and mRNA was markedly increased in both infarcted and non-infarcted left ventricles in a rat model of acute myocardial infarction [16]. Treatment with an angiotensin converting enzyme (ACE) inhibitor suppressed the overproduction of AM in association with improved hemodynamics.

Studies have also shown that AM is an antihypertrophic peptide able to inhibit angiotensin II- and endothelin-1-induced hypertrophy of cultured neonatal cardiac myocytes and fibroblasts [17,18]. AM also inhibits collagen production and proliferation by cardiac fibroblasts, possibly via a cAMP-dependent mechanism [19]. These findings suggest AM can function as an anti-remodeling autocrine and/or paracrine factor in the heart. Consistent with that idea, Nakamura *et al.* showed that continuous infusion of AM has beneficial effects on hemodynamics in a rat model of acute myocardial infarction [20]. They also showed that the infusion of AM improved survival and ameliorated progression of left ventricular remodeling and heart failure with a reduction in left ventricular levels of mRNAs encoding ACE, p22-phox and urinary isoprostane [21]. Thus the beneficial effects of AM administration after acute myocardial infarction include improvement of hemodynamics and reduction of left ventricular remodeling, in part by inhibiting oxidative stress and ACE expression (Fig. **3**).

1.4. Pathophysiological Role of Cardiac AM in Ischemia/Reperfusion

In a rat coronary ligation model, AM significantly attenuated myocardial ischemia/reperfusion injury [22]. AM significantly reduced myocardial infarct size, left ventricular end-diastolic pressure and myocardial apoptotic death. These beneficial effects were almost completely abolished by pretreatment with wortmannin, suggesting the cardioprotective effects of AM are mediated via a phosphatidylinositol 3-kinase (PI3K)/Akt-dependent pathway. Investigators have also shown the effect of local AM gene delivery into the heart on the apoptosis induced by acute ischemia/reperfusion. AM gene transfer increased phosphorylation of Akt and glycogen synthase kinase (GSK-3beta), but reduced GSK-3beta and caspase-3 activities in the heart. The effects of AM on GSK-3beta and caspase-3 activities were blocked by CGRP(8-37) and by an adenovirus harboring dominant-negative Akt [23]. Thus AM may act via the Akt-GSK-caspase signaling pathway to protect against cardiomyocyte apoptosis induced by ischemia/reperfusion injury (Fig. **3**). This cardioprotective effect of AM was confirmed using a heterozygous AM knockout (AM+/-) mouse model [24]. Infarcts elicited by 30 min of regional myocardial ischemia were larger in AM+/- mice than in wild type mice. Moreover, treatment with exogenous recombinant AM prior to coronary occlusion rescued the ischemia-reperfusion intolerant phenotype of AM+/- mice in association with augmented phosphorylation of Akt and eNOS. Nishida *et al.* [25] recently reported that AM treatment for 10 min before ischemia significantly reduced infarct size after ischemia/reperfusion, as compared to control, and that this infarct size-limiting effect of AM was abolished by a mitochondrial Ca^{2+}-activated K^+ channel blocker or by a protein kinase A inhibitor (Fig. **3**). Interestingly, treatment with AM for the first 10 min of reperfusion also significantly reduced infarct size, as compared with control, and this cardioprotective effect of AM was unaffected by a mitochondrial Ca^{2+}-activated K^+ channel blocker, but was abolished by a PI3K inhibitor. This suggests the cardioprotective effects of pre- and post-ischemia treatment with AM in ischemia/reperfusion models are mediated via different signaling pathways. In addition, AM reportedly induces angiogenesis and inhibits apoptosis. Infusion of AM for 3 days together with transplantation of bone marrow-derived mononuclear cells (BMCs) reduced infarct size and improved cardiac function to a greater degree than AM monotherapy in a rat model of myocardial infarction [26]. AM infusion plus BMC transplantation led to greater increases in capillary density than was obtained with AM or BMCs alone, and AM markedly reduced the numbers of apoptotic cells among the transplanted BMCs. Thus the beneficial effect of AM infusion may be mediated in part by the angiogenic properties of AM itself and in part by its anti-apoptotic effect on BMCs.

One recent study addressed the mechanism underlying the beneficial effects of AM in an ischemia/reperfusion model [27]. It was found that AM stimulated NO synthesis,

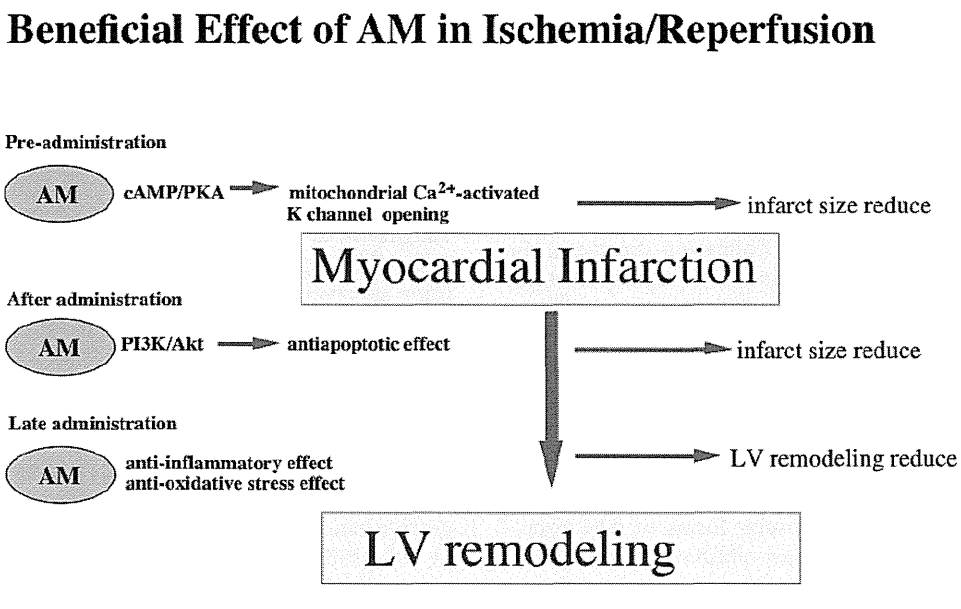

Fig. (3). Mechanism of effect of AM in ischemia/reperfusion.

as indicated by increased NO levels in the coronary effluent throughout reperfusion. AM limited infarct size in association with a 2.45-fold increase in myocardial cGMP after 10 min of reperfusion, and the soluble guanylate cyclase inhibitor ODQ abolished the infarct size-limiting effect of AM. Thus AM may increase the bioavailability of NO in the intact myocardium, and the cytoprotective action of AM against ischemia-reperfusion injury may be mediated via the NO/sGC/cGMP pathway.

1.5. Clinical Application of AM in Acute Myocardial Infarction

The studies summarized above indicate AM reduces infarct size and inhibits myocyte apoptosis, suggesting AM may be a clinically useful peptide. In the first clinical pilot study of intravenous AM in patients with acute myocardial infarction, AM was infused for 12 h at a rate of 0.0125-0.025 μg/kg·min [28]. During the infusion, hemodynamics remained stable in all but two patients. Furthermore, at 3 months, the wall motion index in the infarcted area was significantly better than at baseline, and infarct size evaluated by cardiac magnetic resonance was significantly smaller than the baseline. Thus AM could serve as an adjunct to percutaneous coronary intervention, considering the variety of its potentially protective cardiovascular actions. However, these data are preliminary and will require confirmation in future studies.

2. AM IN HEART FAILURE

2.1. Plasma AM and MR-proAM Levels

Plasma AM levels normally range from 3 to 10 pmol/L (Table **1**), depending on the assay used. Although AM peptide and mRNA are strongly expressed in adrenal gland, heart, kidney and lung, there is normally no step-up in AM levels between the CS, renal vein or adrenal veins and the aorta [29]. Therefore, the source of plasma AM is now thought to be the vasculature, as AM mRNA is strongly expressed in both endothelial cells and vascular smooth muscle cells. Plasma AM levels are increased in heart failure in proportion to the severity of the disease [30-33]. We showed that there is no increase of plasma AM in patients with NYHA class I, but the levels are slightly but significantly elevated in patients with NYHA class II and are increased even further in NYHA classes III and IV (Fig. **4**). Plasma AM levels are positively correlated with plasma ANP, BNP and norepinephrine levels and negatively correlated with left ventricular ejection fraction [30]. Following treatment in cases of heart failure, plasma ANP and BNP rapidly decline, but plasma AM declines more slowly. These results indicate that plasma AM increases in proportion to disease severity and that the mechanism of the increase may be related to the increased plasma volume and/or sympathetic nerve activity. This finding is consistent with another study, which showed a good relationship between plasma AM levels and pulmonary capillary wedge pressure [33].

As mentioned above, two molecular forms of AM circulate in human plasma, and the major circulating form is the inactive form AM-Gly [7,34]. Hirayama *et al.* reported that both forms of AM are similarly increased in patients with heart failure [35], making AM a potentially useful biochemical marker of the severity of heart failure. In addition, plasma AM is an independent prognostic indicator of mild to moderate heart failure [36] and of ischemic heart failure with left ventricular dysfunction [10]. Thus AM is not only a biochemical marker for evaluating the severity of heart failure, it is also a prognostic indicator.

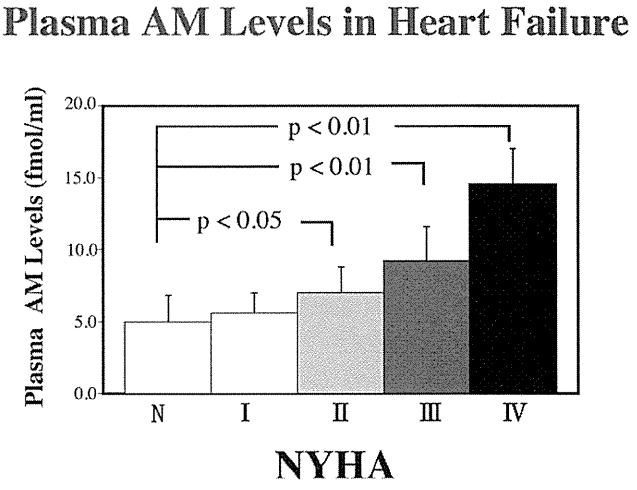

Fig. (4). Plasma AM levels in patients with heart failure in NYHA functional classes I, II, III and IV, and in healthy subjects. In heart failure patients, plasma AM levels tended to be increased in NYHA I, not significantly different from that in normal subjects. However, there was a significant increase of plasma AM levels in patients in NYHA II, III and IV. Thus, graded increase of the plasma levels of AM were observed in patients with heart failure in NYHA functional class as II, III to IV.

MR-proAM was recently investigated in the context of heart failure. Gegenhuber *et al.* [37] compared the abilities of MR-proAM and BNP to serve as a prognostic marker in 137 patients with acute destabilized heart failure. ROC curve analysis showed that the areas under the curve for the prediction of 1-year mortality were similar for BNP and MR-proAM, and Kaplan-Meier curve analyses showed that the predictive values of BNP and MR-proAM for survival probability were comparable. In addition, multivariable Cox proportional hazard analysis revealed that elevated BNP and MR-proAM concentrations were the strongest predictors of mortality, suggesting the predictive properties of MR-proAM may be similar to those of BNP in acute destabilized heart failure. Haehling *et al.* [38] assessed MR-proAM in 501 congestive heart failure patients and showed that it increased with NYHA class. Increasing MR-proAM was a predictor of poor survival at 12 months, and the areas under the ROC curves for MR-proAM and NT-proBNP were similar. Cox proportional hazard analysis showed that both NT-proBNP and MR-proAM added prognostic value to a base model of left ventricular ejection fraction, age, creatinine and NYHA class, though adding MR-proAM to the base model gave stronger prognostic power than adding NT-proBNP. Thus MR-proAM is an independent predictor of mortality in congestive heart failure patients that adds prognostic information to NT-proBNP. Maisel *et al.* [39] assessed the prognostic value of MR-proAM in a 15-center, international study of 1,641 patients presenting to the emergency department with dyspnea. Using cut-off values from ROC analyses, the accu-

racy to predict the 90-day survival of heart failure patients was 73% for MR-proAM and 62% for BNP. In adjusted multivariable Cox regression, MR-proAM, but not BNP, carried independent prognostic value. Thus MR-proAM identifies patients with high 90-day mortality risk and adds prognostic value to BNP.

These results suggest that elevated MR-proAM is associated with an increased risk of mortality and morbidity in patients with heart failure, independent of BNP or NT-proBNP. In fact, MR-proAM may outperform all other established markers in the identification of patients at highest risk of death, particularly death within 30 days. It may thus be useful for AM and/or MR-proAM to be included in the routine clinical workup of patients with heart failure.

2.2. Cardiac AM System in Cardiac Hypertrophy and the Failing Heart

Early studies showed that AM immunoreactivity is increased in the failing heart [40] and in a model of heart failure [41]. AM immunoreactivity is also increased in pressure overload-induced cardiac hypertrophy, and there is a strong correlation between the level of AM immunoreactivity and left ventricular mass [42]. Thus cardiac AM levels are upregulated in the hypertrophic and failing heart in association with an increase in ventricular weight or fetal cardiac gene expression [43]. CS-aortic step-up of plasma AM is enhanced in failing hearts, suggesting cardiac AM production is increased in patients with heart failure [41].

Two molecular forms of AM, its transcript and its heterodimeric receptors (CLR/RAMPs) have been investigated in relation to cardiac hypertrophy and heart failure. Left ventricular expression of the genes encoding AM and its receptor is significantly higher in hypertrophic and failing hearts than in healthy hearts [44,45]. Cardiac tissue levels of AM-m and AM-total are also significantly higher in hypertrophic and failing hearts than in healthy hearts, and the AM-m/AM-total ratio is significantly higher in left ventricular tissue than in plasma. Furthermore, the left ventricular AM-m/AM-total ratio significantly correlates with the left ventricular weight/body weight ratio [45,46]. These results suggest that AM amidating enzyme activity, AM ligand, and its receptor system are all upregulated in severely affected hypertrophic and failing hearts; that is, all components of the cardiac AM system are upregulated in hypertrophic and failing hearts.

To investigate whether the elevated plasma and tissue AM levels seen in hypertrophic and failing hearts exert cardioprotective effects, studies involving adenovirus-mediated AM gene delivery and chronic administration of AM have been carried out. Somatic gene delivery using an adenovirus harboring human AM cDNA under the control of the cytomegalovirus promoter/enhancer significantly reduced left ventricular weight and cardiomyocyte diameter in severely hypertensive rats, while also reducing interstitial fibrosis, extracellular matrix formation and blood pressure [47]. These findings suggest that increasing AM may protect against cardiac remodeling and renal damage due to hypertension.

To investigate the role of endogenous AM in the transition from left ventricular hypertrophy to heart failure, the effects of long-term AM infusion in a rat model of heart failure were studied [48]. Long-term infusion of human AM reduced left ventricular end-diastolic pressure, right ventricular systolic pressure, right atrial pressure and left ventricular weight/body weight ratio without significantly affecting mean arterial pressure. Infusion of human AM significantly reduced endogenous plasma AM levels in rats, and also reduced plasma renin, aldosterone and ANP levels and prolonged survival. These results suggest that endogenous AM plays a compensatory role in heart failure. In addition to these salutary effects of long-term treatment with AM in heart failure, long-term AM attenuates left ventricular remodeling after acute myocardial infarction, in part by reducing oxidative stress and inhibiting ACE expression [20]. A cardioprotective effect of endogenous AM against stress-induced cardiac hypertrophy was investigated using AM+/- mice [49]. Aortic constriction reduced ejection fraction to a greater degree in AM+/- mice than in wild type mice, suggesting that AM and its receptor play an important role in preserving cardiac function. It thus appears that up-regulated cardiac expression of AM and its receptor may be an adaptive and protective response to stresses such as cardiac hypertrophy and heart failure. The cardioprotective effects of AM may be associated with inhibition of the renin-angiotensin-aldosterone system, reduction of oxidative stress, and other factors [20,48,50-52].

2.3. Effect of AM Administration in Experimental Heart Failure

The aforementioned observations indicate that increases in endogenous plasma and tissue AM exert cardioprotective effects in cardiac hypertrophy and heart failure. Those findings, together with the observations that AM has vasodilatory, diuretic and natriuretic effects, and inhibits aldosterone secretion, suggest AM administration may be a useful approach to the treatment of heart failure [53]. Indeed, intravenous infusion of AM reduces calculated peripheral resistance, mean arterial pressure and left atrial pressure, and increases cardiac output in sheep with pacing-induced heart failure [54]. AM also increases urinary sodium, creatinine and cAMP excretion as well as creatinine clearance in conjunction with reduced plasma aldosterone [55]. We also examined the cardiovascular and renal effects of administering two intravenous doses of AM to rats with heart failure [55]. Low-dose AM increased urine flow and urinary sodium excretion without affecting hemodynamic variables. By contrast, high-dose AM reduced mean arterial pressure, right ventricular systolic pressure and right atrial pressure and significantly increased cardiac output in both healthy rats and rats with heart failure. High-dose AM also significantly increased the glomerular filtration rate and renal plasma flow, as well as urine flow and urinary sodium excretion [55]. In addition, treatment with AM for 4 days had pronounced and sustained cardiovascular and renal effects in experimental heart failure, including reductions in cardiac preload and afterload and increases in cardiac output, sodium excretion and glomerular filtration [56]. These results imply that AM is involved in the regulation of blood pressure and volume in heart failure and raises the possibility of its use in the disease's treatment.

Combination therapy with AM plus other drugs such as an ACE inhibitor, a neutral endopeptidase inhibitor or natri-

uretic peptide have been studied, and beneficial and complementary effects of AM and other drugs were reported [57-59].

2.4. Effect of AM Administration to Patients with Heart Failure

The beneficial effects of AM in experimental heart failure in animals strongly suggests that AM administration would be effective in the treatment of human heart failure. We previously examined the acute effects of intravenous infusion of AM (0.05 μg/kg/min) for 30 min on hemodynamic, renal and hormonal responses in patients with heart failure [60]. We found that AM significantly increased the cardiac index, while reducing pulmonary capillary wedge pressure in patients with heart failure and in healthy subjects. AM significantly reduced mean pulmonary arterial pressure only in heart failure patients but increased urine volume and urinary sodium excretion in both groups. Plasma aldosterone fell significantly during and after AM infusion only in the patients. These findings indicate that acute intravenous infusion of AM has beneficial hemodynamic, renal and endocrine effects in patients with heart failure [60]. Lainchbury *et al.* [61] also found that AM infusion significantly reduced mean arterial pressure and left ventricular end-systolic volume and increased cardiac output. Despite the large drop in blood pressure, urine volume, urinary sodium excretion and creatinine clearance were not changed. These results suggest that short-term AM infusion also relieves the symptoms of heart failure and may be therapeutically useful.

We then tested whether long-term administration of AM (0.02 μg/kg/min) + recombinant human atrial natriuretic peptide (hANP) (0.05 μg/kg/min) could be used as a therapeutic regimen in patients with acute decompensated heart failure in a clinical setting [62]. Seven acute decompensated heart failure patients with dyspnea and pulmonary congestion were studied. AM+hANP was infused for 12 h, after which hANP was infused alone for 12 h, and hemodynamic, renal, hormonal and oxidative stress responses were evaluated. AM+hANP significantly reduced mean arterial pressure, pulmonary arterial pressure, and systemic and pulmonary vascular resistance without affecting heart rate, and increased cardiac output over baseline at most of the time-points studied (Fig. **5**). In addition, AM+hANP reduced aldosterone, BNP, and free radical metabolites to levels below those seen at baseline. AM+hANP also increased urine volume and urinary sodium excretion as compared to baseline.

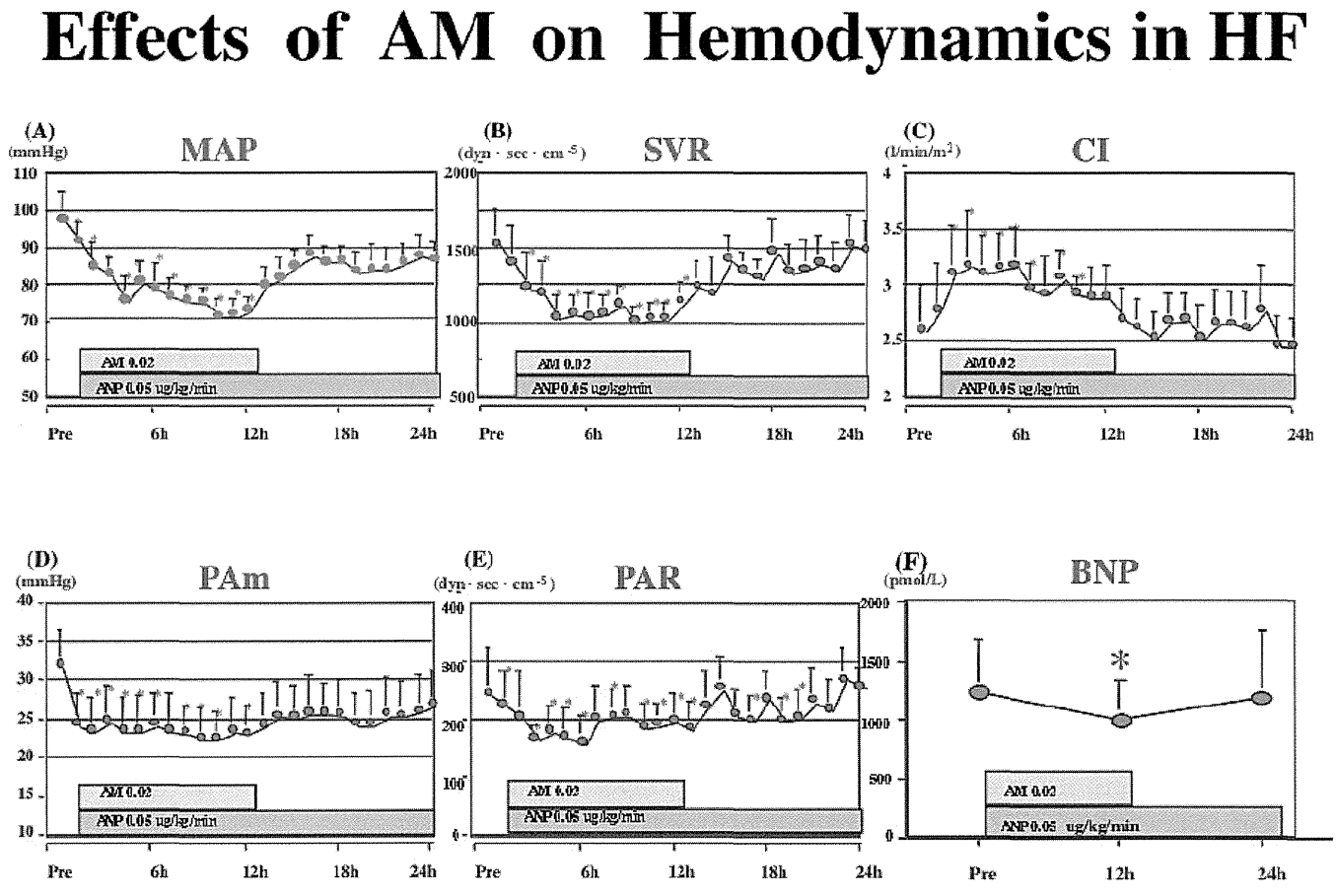

Fig. (5). Effects of long-term AM+hANP combination therapy and hANP monotherapy on mean arterial pressure (MAP)(A), systemic vascular resistance (SVR)(B), cardiac index (CI)(C), mean pulmonary arterial pressure (PAm)(D), pulmonary arterial resistance (PAR)(E) and plasma BNP levels (F) in patients with acute decompensated heart failure. Infusion of AM+hANP significantly decreased MAP, SVR, PAm and PAR and increased CI at most of the time-point compared with the baseline levels. Infusion of AM+hANP also significantly decreased plasma BNP levels. *P<0.05 vs. time 0.

After switching to hANP monotherapy, mean arterial pressure and systemic vascular resistance increased while the cardiac index declined. On the other hand, urine volume and urinary sodium excretion did not change [62]. Although this was a small pilot trial, AM+hANP therapy showed beneficial hemodynamic and hormonal effects in acute decompensated heart failure. Specifically, prominent beneficial effects on systemic and pulmonary vascular resistance and cardiac index were observed. Thus intravenous infusion of AM with hANP could be a useful approach to treating acute decompensated heart failure. That said, these data are preliminary and require confirmation in a larger clinical study. Our working hypothesis for beneficial effects of AM in heart failure is illustrated in Fig. **6**.

3. PULMONARY HYPERTENSION

3.1. Plasma AM Levels in Pulmonary Hypertension

AM and its receptors are strongly expressed in the lung [1,63], which suggests AM is involved in the regulation of the pulmonary circulation, most likely in part through dilation of the pulmonary vasculature and reduction of pulmonary vascular resistance [64]. Yoshibayashi *et al.* [65] reported that plasma AM levels are elevated in young patients with pulmonary hypertension, and that the plasma AM is higher in the pulmonary artery than in the pulmonary vein, suggesting extraction of AM from the pulmonary circulation. We observed that plasma AM is elevated in secondary pulmonary hypertension related to mitral stenosis, and that there are significant relationships between plasma AM levels and mean pulmonary artery pressure, total pulmonary vascular resistance, and pulmonary vascular resistance [66]. In addition, plasma AM levels reportedly correlate with mean right atrial pressure, stroke volume, total pulmonary resistance, mean pulmonary arterial pressure and plasma ANP [67], and increased plasma AM is also seen in systemic sclerosis patients with secondary pulmonary hypertension [68] and chronic obstructive lung disease [69]. Although plasma AM levels were not significantly affected by acute NO inhalation during long-term follow-up in patients with pulmonary hypertension, they were significantly elevated in association with increases in total pulmonary resistance [67]. Taken together, these results indicate that plasma AM levels increase in proportion to the severity of pulmonary hypertension and that AM is extracted from the pulmonary circulation, perhaps in an effort to reduce pulmonary arterial pressure.

These results are consistent with findings from experimental animal studies. Shimokubo *et al.* [70] measured AM levels in plasma and right ventricular tissue from rats with monocrotaline-induced pulmonary hypertension. Both were higher than in control rats, which suggests a role for AM in pulmonary hypertension. In a hypoxia-induced pulmonary hypertension model, plasma AM was significantly elevated on day 21 of exposure to a hypobaric hypoxic environment, and expression of AM mRNA and peptide was increased in the right ventricle, right atrium and left atrium [71].

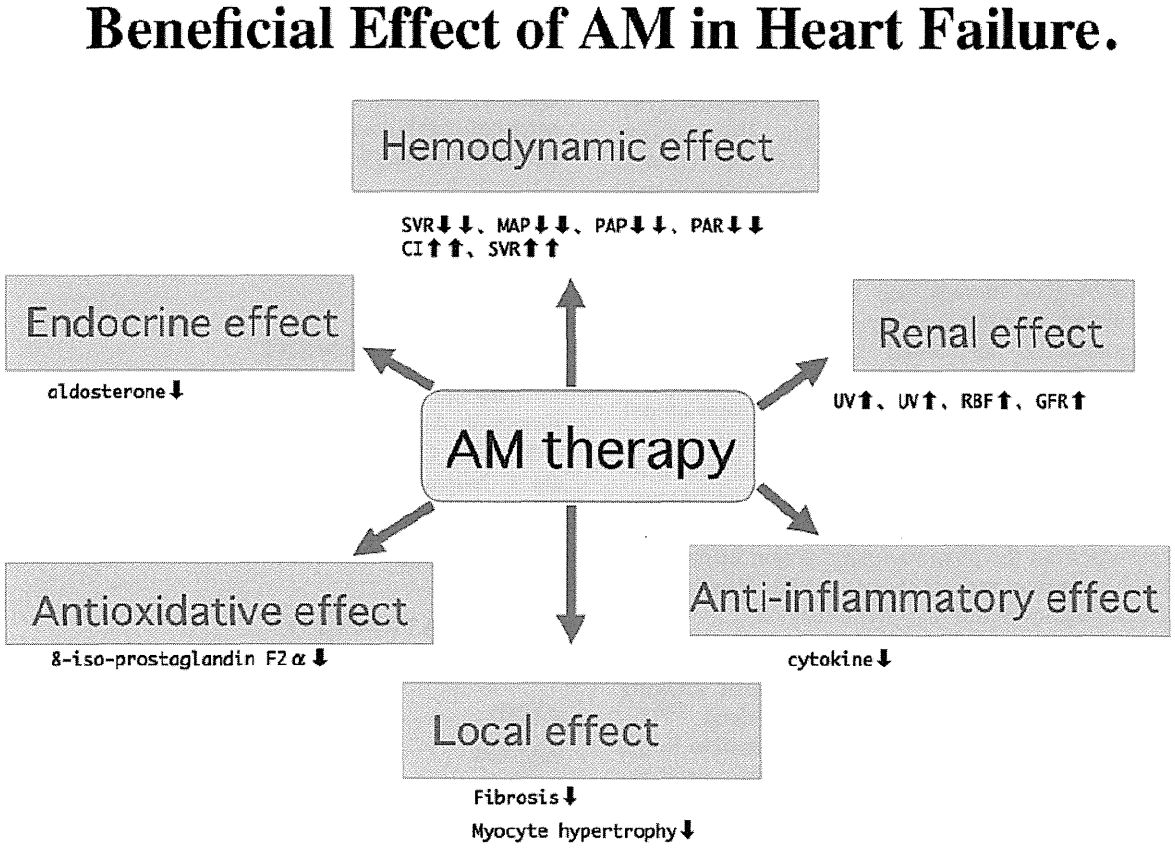

Fig. (6). Our working hypothesis of the mechanism of effect of AM therapy in heart failure.

3.2. Use of AM in the Treatment of Pulmonary Hypertension

Yoshihara *et al.* [72] tested whether chronic infusion of rat AM would affect monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy. Using an osmotic minipump, they subcutaneously infused rats with AM for 21 days, which ameliorated the pulmonary hypertension and right ventricular hypertrophy with a slight increase in plasma AM levels. This suggests AM may be useful for the treatment of pulmonary hypertension. Consistent with that idea, AM also exerts beneficial effects in hypoxia-induced pulmonary hypertension [73,74] and endotoxin-induced pulmonary hypertension models [75]. Matsui *et al.* [74] showed that endogenous AM exerts as a prorotective peptide against hypoxia-induced vascular remodeling, mediated via suppression of reactive oxygen species using heterozygous AM-knockout mouse.

The acute effect of AM on the hemodynamics and hormonal response in patients with pulmonary hypertension has also been evaluated. Nagaya *et al.* [76] demonstrated that intravenous infusion of AM (0.05 μg/kg/min) produced a 44% increase in the cardiac index and a 32% decrease in pulmonary vascular resistance, with a 4% reduction in mean pulmonary arterial pressure and a reduction in plasma aldosterone levels. AM also significantly reduced mean systemic arterial pressure in this study. To avoid the effect of AM on systemic blood pressure, repeated inhalation of aerosolized AM was tested in monocrotaline-induced pulmonary hypertension in rats [77]. Three weeks of AM inhalation significantly reduced pulmonary arterial pressure, total pulmonary resistance and the median wall thickness of peripheral pulmonary arteries without changing systemic blood pressure or heart rate. Aerosolized AM therapy was also tested in cases of human idiopathic pulmonary arterial hypertension [78]. Acute inhalation of aerosolized AM elicited a 13% reduction in mean pulmonary arterial pressure and a 22% reduction in pulmonary vascular resistance without changing the systemic arterial pressure or heart rate. Inhalation of aerosolized AM also increased exercise capacity.

Recently, AM gene therapy was tested in a pulmonary hypertension model. Intratracheal transfer of AM gene using polyplex nanomicelles attenuated monocrotaline-induced pulmonary hypertension in rats [79]. Hybrid cell-AM gene therapy was also tested in a pulmonary hypertension model. Administration of AM gene-transduced endothelial progenitor cells provided significantly greater relief of monocrotaline-induced pulmonary hypertension in rats than untransduced endothelial progenitor cells [80]. Thus a hybrid cell-gene therapy based on the phagocytosing action of endothelial progenitor cells may represent a novel therapeutic strategy for the treatment of pulmonary hypertension.

Another recent study showed that an AM derivative radiolabeled with ^{99m}Tc can be used as a molecular imaging agent to visualize the lung circulation [81]. Indeed, ^{99m}Tc-AM could provide a low-molecular-weight alternative to the ^{99m}Tc-macroaggregated albumin particles currently used for pulmonary embolism diagnosis. Moreover, this radiolabeled AM derivative may facilitate diagnosis and follow-up of pulmonary hypertension, as uptake of ^{99m}Tc-AM by the lungs is greatly reduced in rats with monocrotaline-induced pulmonary hypertension, as compared to control [82]. Thus ^{99m}Tc-labeled AM derivatives are highly promising new tools for examining the pulmonary circulation.

This chapter described the role of AM in the pathophysiology of pulmonary hypertension. The therapeutic potential of AM for the treatment of pulmonary hypertension was also described. Plasma AM levels are significantly elevated in both primary and secondary pulmonary hypertension, and the AM is thought to contribute to a compensatory mechanism. Moreover, exogenous administration of pharmacological levels of AM induces further hemodynamic improvement, which suggests AM administration may be useful in the treatment of patients with pulmonary hypertension. Despite recent therapeutic advances, including the use of prostanoids, endothelin antagonists and phosphodiesterase inhibitors, among others, pulmonary arterial hypertension remains a challenging condition with a poor prognosis. In that context, further investigation of the efficacy of AM in the treatment of pulmonary arterial hypertension would seem warranted.

CONCLUSIONS

We described the current understanding of the role of AM in the pathophysiology of cardiovascular diseases such as acute myocardial infarction, heart failure and pulmonary hypertension. Plasma AM levels are increased in proportion to the severity of these diseases and plasma AM and/or MR-proAM are good prognostic predictors in these conditions. Locally increased AM may exert compensatory effects, which is consistent with exogenous AM's beneficial effects in acute myocardial infarction, heart failure and pulmonary hypertension. Thus AM administration appears to be a promising new approach to the treatment of cardiovascular disease. To confirm the beneficial effects of AM in the treatment of cardiovascular disease, further clinical and basic studies and a larger clinical trial will be required.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATION LISTS

AM	=	Adrenomedullin
cAMP	=	Cyclic adenosine 3',5'-monophosphate
AM-Gly	=	Glycine-extended AM
MR-proAM	=	Mid-regional proAM
CS	=	Coronary sinus
NT-proBNP	=	N-terminal proBNP

ROC	=	Receiver-operating characteristic
ACE	=	Angiotensin converting enzyme
PI3K	=	Phosphatidylinositol 3-kinase
GSK	=	Glycogen synthase kinase
BMCs	=	Bone marrow-derived mononuclear cells
cGMP	=	Cyclic guanosine 3',5'-monophosphate
NO	=	Nitric oxide
NYHA	=	New York Heart Association
BNP	=	Brain natriuretic peptide
hANP	=	Human atrial natriuretic peptide

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Increased Expression of HCN Channels in the Ventricular Myocardium Contributes to Enhanced Arrhythmicity in Mouse Failing Hearts

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Increased Expression of HCN Channels in the Ventricular Myocardium Contributes to Enhanced Arrhythmicity in Mouse Failing Hearts

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Background—The efficacy of pharmacological interventions to prevent sudden arrhythmic death in patients with chronic heart failure remains limited. Evidence now suggests increased ventricular expression of hyperpolarization-activated cation (HCN) channels in hypertrophied and failing hearts contributes to their arrythmicity. Still, the role of induced HCN channel expression in the enhanced arrhythmicity associated with heart failure and the capacity of HCN channel blockade to prevent lethal arrhythmias remains undetermined.

Methods and Results—We examined the effects of ivabradine, a specific HCN channel blocker, on survival and arrhythmicity in transgenic mice (dnNRSF-Tg) expressing a cardiac-specific dominant-negative form of neuron-restrictive silencer factor, a useful mouse model of dilated cardiomyopathy leading to sudden death. Ivabradine (7 mg/kg per day orally) significantly reduced ventricular tachyarrhythmias and improved survival among dnNRSF-Tg mice while having no significant effect on heart rate or cardiac structure or function. Ivabradine most likely prevented the increase in automaticity otherwise seen in dnNRSF-Tg ventricular myocytes. Moreover, cardiac-specific overexpression of HCN2 in mice (HCN2-Tg) made hearts highly susceptible to arrhythmias induced by chronic β-adrenergic stimulation. Indeed, ventricular myocytes isolated from HCN2-Tg mice were highly susceptible to β-adrenergic stimulation-induced abnormal automaticity, which was inhibited by ivabradine.

Conclusions—HCN channel blockade by ivabradine reduces lethal arrhythmias associated with dilated cardiomyopathy in mice. Conversely, cardiac-specific overexpression of HCN2 channels increases arrhythmogenicity of β-adrenergic stimulation. Our findings demonstrate the contribution of HCN channels to the increased arrhythmicity seen in failing hearts and suggest HCN channel blockade is a potentially useful approach to preventing sudden death in patients with heart failure. (***J Am Heart Assoc.*** **2013;2:000150 doi: 10.1161/JAHA.113.000150)**

Key Words: arrhythmia • HCN channel • heart failure • ion channels

Despite recent progress, the efficacy of available pharmacological interventions aimed at preventing lethal arrhythmias associated with chronic heart failure remains limited. Indeed, as many as 50% of deaths among heart failure patients are sudden and unexpected, presumably caused by lethal arrhythmias.[1] Thus, identification of potential therapeutic targets based on knowledge of the molecular mechanism underlying the enhanced arrhythmicity in failing hearts would be highly desirable.

Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels comprise an ion channel family (HCN1-4) that carries a current termed I_f or I_h, which has been recorded in both the heart and nervous system.[2,3] In the healthy adult heart, HCN channels are predominantly expressed in the conduction system, especially in the sinoatrial node, where HCN4 is the major isoform and controls cardiac rhythmicity.[3] HCN channels (HCN1-4) are also expressed in ventricular myocytes, where HCN2 is the dominant isoform, though expression of HCN channels in the healthy adult ventricular myocardium is generally much weaker than in the conduction system, so that I_f currents are rarely detectable in normal

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