**Adrenomedullin: a Possible Autocrine or Paracrine Hormone in the Cardiac Ventricles**

Johji KATO, Toshihiro TSURUDA, Kazuo KITAMURA, and Tanenao ETO

Adrenomedullin (AM), a potent vasodilator peptide originally isolated from pheochromocytoma, is expressed in cardiovascular tissues such as those of the cardiac atria and ventricles. Cell culture experiments have shown that AM peptide is synthesized and secreted from cardiac myocytes and fibroblasts of neonatal rats. Humoral factors, such as angiotensin II (Ang II) and endothelin-1 (ET-1), and mechanical stress due to pressure and volume overload to the heart have been shown to be involved in AM expression of the myocardium in both *in vitro* and *in vivo* studies. The effects of AM on cardiomyocytes and cardiac fibroblasts have been examined in *in vitro* studies, with the result that AM was shown to exert inhibitory actions on myocyte hypertrophy and on proliferation and collagen production of cardiac fibroblasts in an autocrine or paracrine manner. In rats, experimental therapeutic intervention consisting of transfer of the AM gene or of recombinant AM appears to partly inhibit the progression of cardiac hypertrophy and remodeling. It has been shown that the calcitonin receptor-like receptor (CRLR) and receptor-activity-modifying protein (RAMP) act together to function as AM receptors, although in this regard there are a number of issues, including the cellular mechanism of AM actions, that remain to be addressed. In addition, the role of proadrenomedullin N-terminal 20 peptide (PAMP), which is derived from preproAM, is another topic for future experiments. Collectively, the research data accumulating in this area suggest that AM plays a role as an autocrine or paracrine hormone in the cardiac ventricles, and that AM might be utilized as a therapeutic tool in the treatment of hypertensive or ischemic heart disease. (*Hypertens Res* 2003; 26 (Suppl): S113–S119)

**Key Words:** adrenomedullin, autocrine or paracrine hormone, cardiac ventricle

**Introduction**

Various hormonal aspects of adrenomedullin (AM) have been revealed since the discovery of this bioactive peptide by Kitamura *et al.* in 1993 (1, 2). Both AM mRNA and AM peptide are found in various organs, tissues and cells, where AM exerts a wide range of pharmacological effects (2). It has been reported that immunoreactive AM circulates in human blood, and the plasma level of AM has been shown to be elevated in patients with hypertension, acute myocardial infarction, and heart failure (3–8). Analysis of immunoreactive AM in plasma of the coronary sinus has revealed that biologically active AM peptide is secreted from the human heart (9). Previous animal experiments have demonstrated an increase in AM expression in the cardiac ventricles in association with pressure or volume overload to the heart (10–15). These findings suggest that AM plays an important role in the pathophysiology of heart diseases. In this article, we review the relevant literature on the activity of AM as an autocrine or paracrine hormone in the cardiac ventricles.

**AM Production by Cardiomyocytes and Cardiac Fibroblasts**

Following studies in which AM gene expression and AM
peptide were detected in the myocardium (2, 16, 17), Tsuruda et al. examined the production and secretion of AM from cultured cardiac myocytes and fibroblasts isolated from ventricles of neonatal rats (18). As shown in Fig. 1, the cultured cardiomyocytes were positive for immunoreactive AM, and this finding was in agreement with a subsequent immunohistochemical study of the human myocardium (19). By measuring AM levels in the conditioned media, Tsuruda et al. showed that the AM peptide was secreted from cultured cardiomyocytes in a time-dependent manner, and that the preproAM gene was indeed expressed in the cells (18). In the same study, analyses using reverse-phase high performance liquid chromatography (RP-HPLC) showed that the immunoreactive AM secreted from the myocytes was identical to rat AM(1–50), the biologically active form of this peptide (18). According to our previous study, the AM secretion was augmented by angiotensin II (Ang II) and endothelin-1 (ET-1), the peptide hormones involved in cardiac hypertrophy (18, 20, 21). However, the results regarding the effects of ET-1 on AM secretion from myocytes have been inconsistent (20, 22, 23). For example, Autelitano et al. found that ET-1 had an inhibitory effect on AM secretion from cultured cardiac myocytes of rats (23). The reason for these discrepancies remains unknown, although differences in culture conditions or cell-isolation methods may be involved. Similar to cardiomyocytes, cardiac fibroblasts, which make up the interstitium of the myocardium, were also found to express the AM gene at a substantial level and to actively secrete AM peptide (24, 25). Tsuruda et al. found that Ang II and ET-1 augmented the AM production in cultured cardiac fibroblasts, just as they did in cardiomyocytes (25).

Cormier-Regard et al. showed that 1% O2 hypoxia increased the AM gene expression in cultured cardiomyocytes, and suggested that this increase occurred following the heterodimeric binding of α and β subunits of hypoxia-inducible factor-1 (HIF-1) protein to the HIF-1-responsive elements of the promoter region of the AM gene (26, 27). Yoshihara et al. reported that oxidative stress was involved in hypoxia-induced AM secretion from the myocytes, but that hypoxia had no effect on AM secretion from the cardiac fibroblasts (28). According to the report by Tomoda et al., interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and lipopolysaccharide, the potent AM secretagogues for vascular smooth muscle cells, stimulated AM secretion from cardiac fibroblasts, but failed to increase AM secretion from cardiomyocytes (29). These findings indicate not only that the regulatory mechanisms of AM secretion in cardiomyocytes differ from those in cardiac fibroblasts, but also suggest that the disparity in the results described above could be related to the different populations of cultured cardiac cells used.

Cellular Mechanism for AM Production by Cardiomyocytes

As shown by in vivo studies, both preload and afterload to the heart promote cardiac hypertrophy, and at the cellular level, mechanical stress induces hypertrophy of cultured cardiomyocytes (30). Both AM peptide level and AM mRNA expression were found to be augmented in the left ventricle of Dahl salt-sensitive rats with hypertension induced by a high salt diet (10). Ishiyama et al. reported an increased level of AM in the left ventricle of renovascular hypertensive rats, and showed that this increase was closely related to the degree of ventricular hypertrophy (11). Elevated left ventricular AM expression has also been observed in a rat model of volume overload induced by aortocaval shunt (15, 31). Thus, AM production in the cardiac ventricles appears to be stimulated by either pressure or volume overload to the heart. To directly determine whether mechanical stress stimulates AM production in cardiomyocytes in vitro, Tsuruda et al. mechanically stretched the myocytes cultured on silicone dishes with serum-free media (32). Consistent with the in vivo studies, both the AM gene expression and secretion increased in the myocytes stretched mechanically for 24 h (32). Moreover, the stretch-induced increase of AM production was attenuated by an Ang II type 1 (AT1) receptor antagonist, but
not by an AT2 antagonist, suggesting involvement of endogenous Ang II acting through AT1 receptors (32). It is of interest to compare this result to the report by Yamazaki et al. showing that locally produced Ang II mediates stretch-induced hypertrophy of cardiac myocytes through an AT1 receptor subtype (33).

The renin-angiotensin system is involved in the process of cardiac hypertrophy both at the systemic and the local level. As discussed above, Ang II has been shown to be a humoral factor stimulating AM production in cultured cardiomyocytes (21, 22, 32). It has also been reported that quinapril, an angiotensin-converting enzyme (ACE) inhibitor, suppressed ventricular AM expression induced by aortic binding in rats, but that hydralazine had no inhibitory effect on the AM induction despite its reduction of blood pressure (13). Yamakawa et al. found that candesartan, an AT1 receptor antagonist, effectively suppressed ventricular AM expression in a rat model of volume overload induced by aortocaval shunt (31). In their experiment, manidipine, a calcium channel blocker, failed to inhibit the AM induction after 6 weeks of treatment, in spite of the similar reductions of blood pressure and left ventricular end-diastolic pressure, compared with those of the candesartan-treated group (31). These in vivo studies suggest the importance of the renin-angiotensin system in the expression of AM in the heart.

In addition to the renin-angiotensin system, other factors may need to be taken into account as the mechanism modulating AM expression of the myocardium. According to a report by Tsuruda et al., Ang II-induced AM secretion from cultured cardiomyocytes was inhibited completely by the AT1 antagonist, but its inhibitory effect on stretch-induced secretion was partial, suggesting involvement of mechanisms other than Ang II (32). Indeed, Romppanen et al. showed an ET-1- and Ang II-independent induction of AM gene expression in rat cardiac ventricles following a vasopressin-induced pressure overload (34). It seems beyond doubt that the renin-angiotensin system is involved in modulating the AM production in the cardiac ventricles, but the detailed mechanisms, including the involvement of other humoral factors or direct mechanical forces, remain to be clarified.

Both the intracellular Ca2+ signaling system and protein kinase C (PKC) are essential in inducing cardiac hypertrophy (35). Tsuruda et al. examined the effects of the agonists and antagonists of these intracellular signaling systems on AM production and secretion from cultured cardiomyocytes (21). AM secretion was found to be stimulated by phorbol-12-myristate-13-acetate (PMA), a PKC agonist, and by a Ca2+ ionophore or Ca2+ channel agonists. Conversely, the PMA-induced AM secretion was suppressed by an inhibitor of PKC or by depletion of PKC. The Ang II-induced AM secretion was similarly reduced by the PKC inhibitor or by a Ca2+ channel antagonist and a chelator of intracellular Ca2+ (21). Moreover, the calmodulin inhibition by W-7 resulted in suppression of the AM secretion induced by Ang II. It has been reported that both Ca2+/calmodulin-dependent kinase class II and calcineurin are among the key enzymes downstream of calmodulin inducing cardiac hypertrophy (36, 37). The specific inhibitors for these enzymes also attenuated the stimulatory effect of Ang II on the AM secretion from cultured cardiomyocytes (21). Collectively, these results suggest that PKC and the Ca2+-dependent signaling system participate in AM production by cardiac myocytes.

### Growth Inhibition of Cardiomyocytes and Cardiac Fibroblasts by AM

Cardiac hypertrophy has been shown to be an important risk factor for cardiovascular diseases, although the detailed mechanisms of cardiac hypertrophy remain to be clarified. The in vivo data suggest a close association between cardiac hypertrophy and AM production in the myocardium (10–15). For example, Ang II and mechanical stress, both of which are involved in cardiac hypertrophy, have been shown to stimulate AM expression in the left ventricle (11–13, 31). Accordingly, Tsuruda et al. examined the effect of AM on hypertrophy of cultured cardiomyocytes isolated from cardiac ventricles of neonatal rats (18). When evaluated by phenylalanine uptake, Ang II- or fetal bovine serum-stimulated de novo protein synthesis was significantly reduced by synthetic AM (18). Moreover, as shown in Table 1, the morphometrical measurement of cell size revealed that AM inhibited the Ang II-induced enlargement of the myocytes.

Luodonpaa et al. reported that AM inhibited Ang II-induced gene expressions of atrial and brain natriuretic peptides (ANP and BNP), biochemical markers of cardiac hypertrophy, in cultured cardiomyocytes, but that AM had no

### Table 1. Effect of AM on Ang II-Induced Enlargement of Cultured Cardiomyocytes

<table>
<thead>
<tr>
<th></th>
<th>Cell area (µm²)</th>
<th>Perimeter (µm)</th>
<th>Maximal axis (µm)</th>
<th>Minimal axis (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,011 ± 503</td>
<td>240 ± 82</td>
<td>56 ± 16</td>
<td>23 ± 9</td>
</tr>
<tr>
<td>Ang II</td>
<td>1,783 ± 641**</td>
<td>316 ± 87**</td>
<td>66 ± 14**</td>
<td>35 ± 10**</td>
</tr>
<tr>
<td>Ang II + AM</td>
<td>1,142 ± 377**</td>
<td>254 ± 65**</td>
<td>58 ± 13**</td>
<td>26 ± 8**</td>
</tr>
</tbody>
</table>

Cultured cardiomyocytes isolated from ventricles of neonatal rats were incubated with serum-free media in the presence or absence of 10-8 mol/l Ang II or AM. After incubating for 24 h, cell size was measured morphometrically with the National Institutes of Health (NIH) program. Data are presented as means ± SD of 100 cells examined. **p < 0.01, compared with control myocytes and p < 0.01, compared with cells incubated in the presence of Ang II without AM.
effect on the expression of these genes induced by ET-1 or phenylephrine (Phe) (22). On the other hand, Autelitano et al. showed that AM inhibited the Phe-induced hypertrophy of cardiac myocytes (23). The precise reason for this discrepancy is unknown, although it may be related to the different treatment durations or concentrations of AM or agonists used. In any case, the AM concentrations used in these in vitro experiments were higher than those in the conditioned media of cultured cells or in the myocardial tissue (17, 18, 24, 38). To clarify the role of endogenous AM, Tsuruda et al. utilized anti-AM monoclonal antibody to neutralize the action of AM secreted from cultured myocytes (18). Blockade of AM actions by the antibody resulted in an increase in the basal and Ang II-stimulated phenylalanine incorporation into the cells, suggesting an inhibitory effect of endogenous AM on cardiomyocyte hypertrophy (18).

Cardiac fibroblasts in the interstitium of the myocardium also have an important role in the process of cardiac hypertrophy and remodeling (39). Either excessive fibroblast proliferation or increased deposition of collagen, which is synthesized by cardiac fibroblasts, may result in increased myocardial stiffness. The active production and secretion of AM from cardiac fibroblasts led us to speculate that AM is involved in modulating fibroblast proliferation or collagen production by these cells (24, 25). Tsuruda et al. reported an inhibitory effect of AM on the DNA and protein syntheses of cultured cardiac fibroblasts isolated from ventricles of neonatal rats (25). This inhibitory effect was observed in the basal condition as well as in the cells stimulated by Ang II or ET-1 (25). In addition, Horio et al. found that AM suppressed proline uptake of cultured cardiac fibroblasts, a result indicative of inhibition of collagen synthesis by AM (40). Similar inhibition of the DNA synthesis or proline uptake has been observed in cardiac fibroblasts treated with 8-bromo-adenosine 3',5' cyclic monophosphate (cAMP), forskolin or a cAMP-specific phosphodiesterase inhibitor (25, 40). Moreover, in the experiments by Tsuruda et al., when the action of endogenous AM was neutralized by anti-AM monoclonal antibody, the basal level of intracellular cAMP was decreased with a concomitant increase of the basal DNA synthesis (25). This suggests that the endogenous AM secreted from cardiac fibroblasts indeed inhibits their own proliferation via an elevation of the intracellular cAMP level.

Collectively, the results of experiments with cultured cardiac cells suggest that AM participates in the mechanism counteracting cardiac hypertrophy and remodeling as an autocrine or paracrine factor of the myocardium. At the same time, because most of these data were obtained with in vitro studies, we should carefully test this hypothesis by further experiments, preferably in vivo studies. Thus far, the reports on experimental therapeutic intervention by AM would seem to be encouraging. Chao et al. reported the beneficial effect of adenovirus-mediated human AM gene transfer in various rat models of hypertension, such as deoxycorticosterone acetate-salt (DOCA-salt), two-kidney, one-clip renovascular and Dahl salt-sensitive hypertensive rats (41–43). Irrespective of the type of hypertension model used, their data were consistent, i.e., AM overexpression ameliorated cardiac hypertrophy and myocardial fibrosis. These same studies demonstrated that the exogenous human AM gene was expressed in the myocardium of all rat models tested (41–43), although it was unclear whether AM ameliorated cardiac hypertrophy and fibrosis directly via its action on the myocardium or indirectly via a reduction of blood pressure. Recently, Nakamura et al. examined the effect of long-term infusion of recombinant human AM on ventricular remodeling following myocardial infarction of rats (44). When infused intravenously for 4 weeks, AM effectively inhibited myocyte hypertrophy and collagen volume in the non-infarct area of the left ventricle, reducing left ventricular end-diastolic pressure, in a rat model of myocardial infarction (44). These in vivo data suggest not only a role of AM in acting against the progression of cardiac hypertrophy and remodeling, but also the possible applicability of this cardiac hormone as a therapeutic tool for hypertensive and ischemic heart diseases.

**AM Receptors and Their Regulation in the Myocardium**

Many of the actions of AM have been shown to be mediated by intracellular cAMP; for example, AM was found to stimulate cAMP production in cultured vascular endothelial and smooth muscle cells (45, 46). At the same time, it has been shown that the nitric oxide (NO)-guanosine 3',5' cyclic monophosphate (cGMP) pathway also contributes to the actions of AM in rabbit cardiomyocytes (47), and intracellular signaling other than that of the cAMP and NO-cGMP pathways has been suggested to contribute to the actions of AM in the myocardium (48). McLatchie et al. cloned three types of receptor-activity-modifying proteins (RAMP-1 to 3), accessory proteins that act together with the calcitonin-receptor-like receptor (CRLR) to function as AM or CGRP receptors (49). CRLR coexpressed with RAMP-1 serves as a calcitonin gene-related peptide (CGRP) receptor, while it functions as an AM receptor when coexpressed with either RAMP-2 or 3 (49). Previously, Owji et al. extensively examined AM binding sites in rat tissues, and showed the presence of abundant AM binding in the heart (50). In agreement with these findings, Mishima et al. found that all the receptor components of CRLR and RAMP-1 to 3 were expressed in cultured cardiac myocytes of neonatal rats (20).

To better understand the role of AM in the myocardium, it will be essential to identify the specific receptors mediating AM activities. Several independent researchers have shown that the CRLR-RAMP system is linked to adenylate cyclase increasing intracellular cAMP (49, 51, 52). As mentioned earlier in this article, the anti-proliferative action of AM on cardiac fibroblasts appears to be mediated at least in part by cAMP (25, 40); however, there is currently little information about the roles of the CRLR-RAMP system in the inhibitory
effect on cardiomyocyte hypertrophy or in activation of the NO-cGMP signaling system of the heart.

In order to understand the pathophysiological role of AM in heart diseases, it will also be important to clarify the mechanism regulating the expression and function of AM receptors. Totsune et al. reported that expression of the CRLR and RAMP-2 genes, as well as the production of AM, was augmented in rats with congestive heart failure induced by coronary ligation (53). Using the quantitative PCR method, Mishima et al. showed that ET-1, a locally-acting factor involved in the pathophysiology of heart failure, up-regulates the gene expressions of CRLR and RAMP-3, thereby enhancing the intracellular cAMP response to AM (20). These findings suggest a possible modulation of the expression and function of AM receptors by humoral or mechanical factors in the myocardium; at present, however, the available data on this matter are limited. In future studies, it should be possible to gather more information about the regulation of CRLR and RAMP gene expression by analyzing the promoter regions of the genomic DNA for these receptor components.

Is PAMP Another Locally-Acting Hormone in the Heart?

Proadrenomedullin N-terminal 20 peptide (PAMP) is a bioactive peptide derived from the N-terminal portion of the proAM peptide, the precursor of AM (2, 54). Immunoreactive PAMP has been detected in the cardiac ventricles of rats and humans (55, 56), and PAMP was found to be secreted from cultured cardiomyocytes and cardiac fibroblasts (57). Much like AM secretion, the PAMP secretion from these cardiac cells was augmented by growth-promoting factors such as Ang II or fetal bovine serum (57). In addition, the PAMP level in hypertrophied cardiac ventricles of spontaneously hypertensive rats has been shown to be higher than that of normotensive Wistar-Kyoto rats (58). These findings led us to speculate that PAMP, in addition to AM, is involved in cardiac hypertrophy or fibrosis. However, according to our preliminary observations, PAMP failed to affect de novo protein synthesis of the cardiomyocytes and DNA synthesis of the cardiac fibroblasts. PAMP was found to inhibit catecholamine release from the sympathetic nerve endings and the adrenal medullary cells (59, 60), while the role of PAMP in the heart remains unclear and should be further investigated.

Conclusions

The research data accumulating in this field indicate that AM participates, as an autocrine or a paracrine hormone, in the mechanism counteracting the progression of cardiac hypertrophy and remodeling. AM appears to exert a beneficial action on the heart, suggesting that this bioactive peptide might be utilized as a therapeutic tool. At the same time, there are a number of important issues, such as identification of the receptors that mediate AM activities and the intracellular signaling system, that should be addressed in future studies.

References


