CARDIOVASCULAR MEDICINE

Increased pericardial fluid concentrations of the mature form of adrenomedullin in patients with cardiac remodelling

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Background: There is evidence that adrenomedullin has autocrine or paracrine activities that oppose cardiac remodelling. However, it remains unclear whether it exerts those local functions in heart failure patients.

Objective: To investigate the relation between plasma and pericardial fluid concentrations of adrenomedullin and left ventricular haemodynamic variables.

Design: Samples of plasma and pericardial fluid were obtained from 50 patients undergoing cardiac surgery. They were classified into two groups: group N (n = 27) with a left ventricular end diastolic volume index (LVEDVI) ⩽ 90 ml/m^2; and group R (n = 23) with LVEDVI > 90 ml/m^2. Plasma and pericardial fluid concentrations of total adrenomedullin (tAM) and mature adrenomedullin (mAM) were measured and related to the preoperative haemodynamic variables.

Results: Pericardial fluid concentrations of mAM were much higher than the plasma concentration in both group N and group R (mean (SEM), 10.6 (1.7) *v* 3.3 (0.2) fmol/ml, p = 0.0001; and 21.2 (2.8) *v* 3.9 (0.3) fmol/ml, p < 0.0001, respectively). The ratio mAM/tAM in pericardial fluid was significantly higher than in plasma (0.56 (0.02) *v* 0.28 (0.02), p < 0.0001). Pericardial fluid concentrations of mAM, but not plasma concentrations, were significantly correlated with LVEDVI, left ventricular end systolic volume index, left ventricular ejection fraction, and left ventricular mass index (r = 0.60, 0.63, –0.54, and 0.47, respectively).

Conclusions: Raised pericardial fluid concentrations of mAM may reflect the actions of adrenomedullin as a local mediator against cardiac remodelling in patients with left ventricular dysfunction.

Adrenomedullin, originally isolated from human phaeochromocytoma, is a potent vasodilating peptide that also has natriuretic and diuretic actions.[1 2] Human adrenomedullin consists of 52 amino acid residues that have a ring structure formed by an intramolecular disulphide bridge and a tyrosine amide at its C-terminal residue,[1] both of which are essential for hypotensive and other biological activities.[3] In the biosynthesis of adrenomedullin, an intermediate form—adrenomedullin(1-52)-glycine-COOH (iAM)—is processed from proadrenomedullin and subsequently converted to a biologically active mature form, adrenomedullin(1-52)-NH_2 (mAM), by enzymatic amidation.[4] Therefore, although total immunoreactive adrenomedullin (tAM) represents not only mAM but also iAM, only mAM exerts significant biological activities.[5] Recently, it was reported that plasma concentrations of both mAM and iAM were progressively increased in proportion to the severity of heart failure.[6 7]

However, there is also increasing evidence of autocrine or paracrine modes of action of adrenomedullin in various organs[8] including the heart.[9–14] Recent reports suggested that adrenomedullin secreted from cardiac myocytes and fibroblasts may exert its biological effects to counteract pressure and volume overload of the heart in rat models.[10–14] However, there have been no clinical studies on how adrenomedullin of cardiac origin exerts its local pathophysiological functions in the process of cardiac remodelling in failing hearts.

Our aims in the present study were to measure plasma and pericardial fluid concentrations of tAM and mAM in patients undergoing cardiac surgery, and to investigate their relations with haemodynamic variables in cardiac remodelling.

METHODS

Patients

We enrolled 50 consecutive patients (34 men and 16 women) who underwent cardiac surgery at Kyoto University Hospital and Takeda Hospital. There were no exclusion criteria except failure to obtain informed consent. The mean age of the patients was 65.8 years (range 46–87 years). All were clinically evaluated before operation by echocardiography and cardiac catheterisation. They underwent M mode and cross sectional echocardiography and single or biplane left ventriculography according to standard techniques. Left ventricular mass was calculated from the Penn convention according to the equation of Devereux and Reichek,[15] and indexed to body surface area, forming the left ventricular mass index (LVMI). Left ventricular end diastolic and end systolic volume indices (LVEDVI and LVESVI) and left ventricular ejection fraction (LVEF) were calculated from left ventricular cineangiography in the right anterior oblique projection.[16] Left ventricular end diastolic pressure (LVEDP) was also measured. The patients were classified into two groups depending on their LVEDVI values. Group N (the "normal" group) consisted of 27 patients with an LVEDVI of ⩽ 90 ml/m^2; group R (the "remodelling" group) consisted of 23 patients with an LVEDVI of > 90 ml/m^2.

Abbreviations: iAM, intermediate form of adrenomedullin; LVEDP, left ventricular end diastolic pressure; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVMI, left ventricular mass index; mAM, mature adrenomedullin; tAM, total immunoreactive adrenomedullin

Table 1 Clinical characteristics of the patients in group N (no remodelling) and group R (remodelling)

Characteristic	Group N (n=27)	Group R (n=23)	p Value
Age (years)	65 (2)	66 (2)	0.67
Sex (male/female)	20/7	14/9	0.32
NYHA functional class			0.0063
I	11	2	
II	9	8	
III	6	10	
IV	1	3	
Diagnosis			
Coronary artery disease	17	14	0.88
Valvar heart disease	7	12	0.057
Thoracic aortic disease	4	0	0.11
Other	1	0	>0.9999
Comorbidities			
Hypertension	14	15	0.34
Renal failure	6	6	0.19
Positive CRP	4	1	0.36
Diabetes mellitus	10	8	>0.9999
Unstable angina	0	3	0.090
Acute myocardial infarction	1	2	0.59
Old myocardial infarction	3	9	0.021
Haemodynamic variables			
Heart rate (beats/min)	72 (3)	78 (3)	0.10
MAP (mm Hg)	89 (2)	89 (3)	0.85
LVEDVI (ml/m^2)	65 (3)	121 (5)	<0.0001
LVESVI (ml/m^2)	24 (3)	71 (5)	<0.0001
LVEF (%)	66 (3)	42 (3)	<0.0001
LVMI (g/m^2)	141 (9)	193 (14)	0.0017
LVEDP (mm Hg)	12 (1)	15 (2)	0.097
Preoperative drug use			
Heparin infusion	2	3	0.65
β Blockers	4	4	0.80
ACE inhibitors	12	11	0.81

Values are n or mean (SEM).
ACE, angiotensin converting enzyme; CRP, C reactive protein; LVEDP, left ventricular end diastolic pressure; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVMI, left ventricular mass index; MAP, mean aortic pressure; NYHA, New York Heart Association.

All the patients gave their written informed consent. The study protocol was approved by the ethics committees on human research of both Kyoto University Hospital and Takeda Hospital.

Sampling of plasma and pericardial fluid

Blood and pericardial fluid samples were obtained during operation from all the patients. With the exception of β blockers, all oral drug treatment was all discontinued 12–18 hours before surgery. Immediately after incision of the pericardium, undiluted pericardial fluid was collected before heparinisation, except in a few patients with unstable angina who had a continuous heparin infusion. At the same time, blood was drawn from the radial arterial line. These samples were immediately transferred into chilled sterile tubes containing disodium EDTA (1 mg/ml) and aprotinin (500 U/ml). They were centrifuged immediately at 2500 ×*g* for 15 minutes at 4°C. The clarified plasma and pericardial fluid samples were frozen and stored at −80°C, and thawed just before immunoradiometric assay.

Measurement of tAM and mAM in plasma and pericardial fluid

The measurement of tAM and mAM in plasma and pericardial fluid was performed by immunoradiometric assay using a specific kit for each form (adrenomedullin RIA Shionogi, adrenomedullin mature RIA Shionogi; Cosmic Corporation, Tokyo, Japan). These kits were designed to follow the methods developed by Ohta and colleagues.[7 17] These investigators reported that no cross reactivity was observed with partial fragments of adrenomedullin or other peptides similar to adrenomedullin in either assay, and that iAM was not detected in the mAM assay.

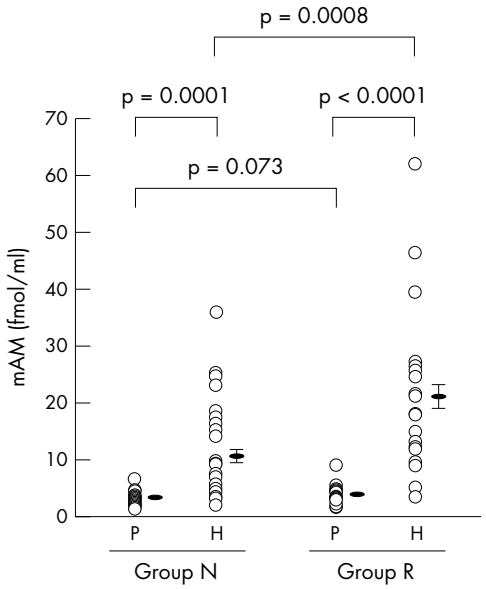

Figure 1 Plasma and pericardial fluid concentrations of mature adrenomedullin (mAM) in groups N and R. Group N, patients with a left ventricular end diastolic volume index of ⩽ 90 ml/m^2; group R, patients with a left ventricular end diastolic volume index of > 90 ml/m^2; H, pericardial fluid; P, plasma.

Statistical analyses

Numerical data are expressed as mean (SEM). Proportion analysis between groups N and R was made by a χ^2 test or Fisher's exact test. Comparisons of variables between the two groups were made by Student's unpaired *t* test or the Mann–Whitney U test. Comparisons of concentrations among each group were performed by Wilcoxon's signed rank test. Multiplicity for statistical tests was adjusted by Bonferroni's method. Student's paired *t* test and Spearman's correlation coefficients were used in assessing the ratio of the mAM concentration to the tAM concentration. Spearman's correlation coefficients were also used to evaluate the relations between adrenomedullin concentrations and preoperative haemodynamic variables. A probability value of $p < 0.05$ was considered significant.

RESULTS

Patient characteristics

Table 1 shows clinical profiles of the study patients in groups N and R. No differences were observed in age or sex. There were no significant differences between the two groups with regard to the proportions of patients who had hypertension, renal failure, positive serum concentrations of C reactive protein, diabetes mellitus, unstable angina, or the acute phase of myocardial infarction. Group R contained significantly more patients with a history of myocardial infarction than group N. There were no differences between the groups in the preoperative use of β blockers or angiotensin converting enzyme (ACE) inhibitors, both of which have actions against cardiac remodelling.

Plasma and pericardial fluid concentrations of tAM

Pericardial fluid concentrations of tAM were higher than the plasma concentrations in group R (38.2 (4.5) *v* 18.7 (2.3) fmol/ml, p = 0.0001), while there were no significant differences between pericardial fluid and plasma levels in group N (18.6 (2.8) *v* 12.7 (1.3) fmol/ml, p = 0.093). There were no differences in plasma tAM concentrations between

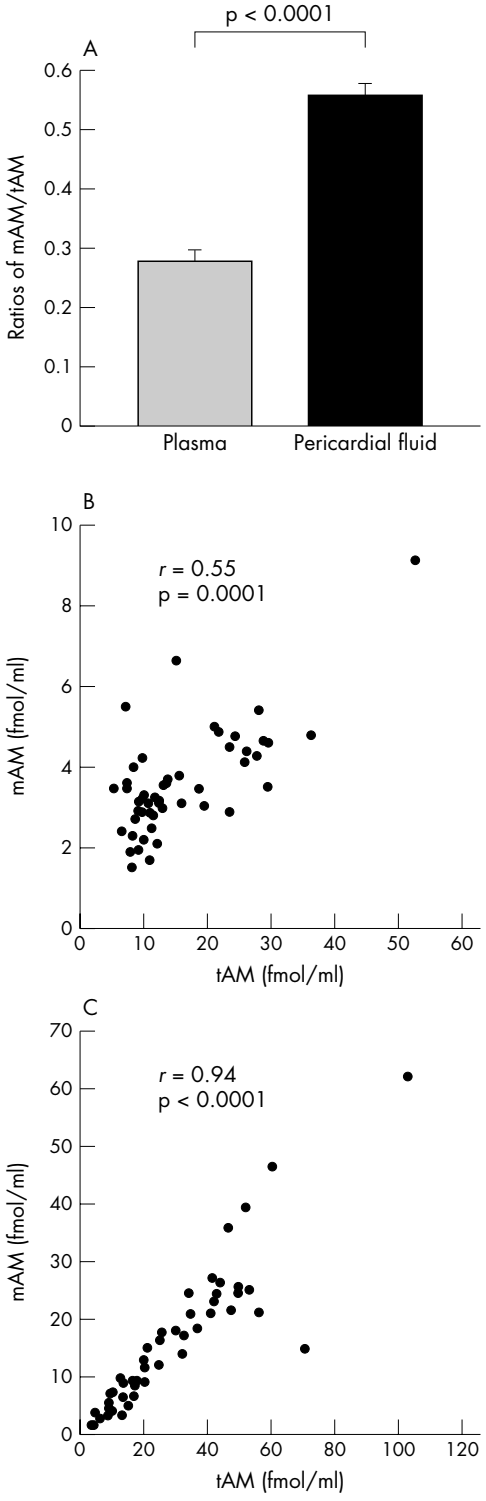

Figure 2 The ratio of the mature form of adrenomedullin (mAM) to total immunorective adrenomedullin (tAM) in plasma and pericardial fluid (A), and the correlations between tAM and mAM coconcentrations in plasma (B) and pericardial fluid (C). *r*, Spearman's correlation coefficient.

the two groups (p = 0.055), but pericardial fluid tAM concentrations were higher in group R than in group N (p = 0.0002).

Plasma and pericardial fluid mAM concentrations

Pericardial fluid mAM concentrations were much higher than the plasma concentrations in both group N and group R (respectively, 10.6 (1.7) *v* 3.3 (0.2) fmol/ml, p = 0.0001; and 21.2 (2.8) *v* 3.9 (0.3) fmol/ml, p < 0.0001). While there were no significant differences between the two groups in plasma

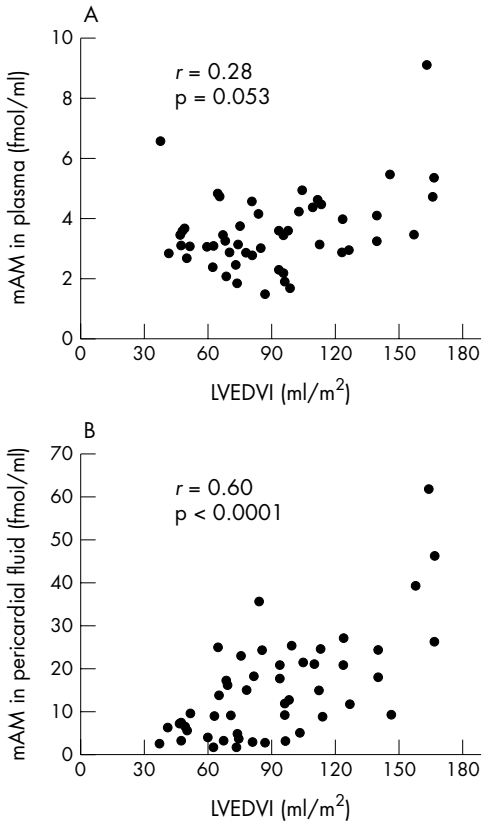

Figure 3 Correlations between left ventricular end diastolic volume index (LVEDVI) and concentrations of the mature form of adrenomedullin (mAM) in plasma (A) and pericardial fluid (B). *r*, Spearman's correlation coefficient.

mAM (p = 0.073), pericardial fluid mAM was higher in group R than in group N (p = 0.0008) (fig 1).

Ratio of mAM to tAM in plasma and pericardial fluid

The ratio of mAM to tAM concentrations in pericardial fluid was significantly higher than in plasma (0.56 (0.02) *v* 0.28 (0.02), p < 0.0001) (fig 2A). Analysis using Spearman's correlation coefficient showed a moderate correlation between these concentrations in plasma and a close correlation in pericardial fluid (fig 2B and 2C). The proportional distribution of the plots in fig 2C shows that the ratios in pericardial fluid are almost constant in this patient group.

Relations of plasma and pericardial fluid tAM and mAM concentrations to haemodynamic variables

Table 2 shows correlations between plasma and pericardial fluid tAM and mAM concentrations and the left ventricular haemodynamic variables. There were no correlations with age, heart rate, or mean aortic pressure. Pericardial fluid concentrations of tAM and mAM were significantly correlated with LVEDVI (tAM, $r = 0.60$; mAM, $r = 0.60$), LVESVI (tAM, $r = 0.66$; mAM, $r = 0.63$), and LVMI (tAM, $r = 0.47$; mAM, $r = 0.47$), while plasma tAM and mAM concentrations were poorly correlated with those variables (table 2; fig 3A and 3B). Significant inverse correlations with LVEF were shown in pericardial fluid concentrations of tAM and mAM ($r = -0.59$, -0.54, respectively). Plasma concentrations of tAM also showed a mild inverse correlation with LVEF. No variables were correlated with LVEDP. Thus the concentrations of tAM and mAM in pericardial fluid were more closely correlated with left ventricular haemodynamic variables than the concentrations in plasma.

DISCUSSION

It was shown very recently that immunoreactive adrenomedullin in human plasma consists of two molecular forms: mAM

Table 2 Correlations of plasma and pericardial fluid concentrations of total immunoreactive adrenomedullin (tAM) and mature form adrenomedullin (mAM) with haemodynamic variables

	tAM in plasma		tAM in pericardial fluid		mAM in plasma		mAM in pericardial fluid	
Variable	*r*	p Value	*r*	p Value	*r*	p Value	*r*	p Value
Age (years)	–0.094	0.51	0.004	0.98	0.15	0.31	–0.065	0.65
Heart rate (beats/min)	0.20	0.17	0.22	0.12	0.088	0.54	0.12	0.42
MAP (mm Hg)	–0.13	0.35	–0.014	0.92	0.10	0.47	–0.034	0.81
LVEDVI (ml/m^2)	0.35	0.016	0.60	<0.0001	0.28	0.053	0.60	<0.0001
LVESVI (ml/m^2)	0.41	0.0043	0.66	<0.0001	0.25	0.078	0.63	<0.0001
LVEF (%)	0.41	0.0044	–0.59	<0.0001	–0.19	0.19	–0.54	0.0001
LVMI (g/m^2)	0.38	0.0082	0.47	0.0010	0.36	0.012	0.47	0.0011
LVEDP (mm Hg)	0.18	0.22	0.27	0.064	0.081	0.57	0.16	0.22

LVEDP, left ventricular end diastolic pressure; LVEDVI, left ventricular end diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end systolic volume index; LVMI, left ventricular mass index; MAP, mean aortic pressure.

and iAM.[5] Although various reports on the quantification of plasma adrenomedullin have been published,[18–22] few have focused on mAM in relation to heart failure.[6 7] However, because the cyclic structure formed by a disulphide bond and the amidated C terminal residue of the adrenomedullin molecule are critical for its receptor binding and biological activities,[3] iAM is considered to have much lower biological activity than mAM. In preliminary data, the vasodilator activity of iAM was only 5% of the activity of mAM.[5] Thus, as tAM may not necessarily reflect all the activities of adrenomedullin, quantification of mAM should be performed for a full understanding of the pathophysiological role of adrenomedullin.

We and other investigators have measured pericardial fluid concentrations of various substances in cardiac patients, and some were greatly increased in comparison with the plasma concentrations.[23–26] It is generally considered that pericardial fluid is not merely an ultrafiltrate of plasma, but also a transudate from the cardiac interstitium.[27] In addition, a recent study suggested that the pericardium may itself release active cardiovascular mediators that function in a paracrine fashion.[28] Therefore it seems possible that pericardial fluid contains higher concentrations of biologically active substances that exert local functions in the heart than does plasma. Furthermore, adrenomedullin has a molecular weight of about 6 kDa,[1] which is well below the molecular weight limit for large molecules to diffuse from the cardiac interstitium into the pericardial space.[27] We considered such issues when we determined the concentrations of mAM in pericardial fluid in comparison with plasma in this study.

There have been several reports that plasma concentrations of tAM are raised in patients with congestive heart failure.[18–20] It has also recently been shown that plasma concentrations of mAM and iAM increase progressively with deterioration in heart failure.[6 7] In our data, however, differences in plasma concentrations of tAM and mAM between group N and group R did not reach significance. This discrepancy may reflect our selection of patients for study, where none was excluded irrespective of the presence of comorbidities that might affect plasma adrenomedullin concentrations—for example, hypertension, renal failure, inflammatory reactions, and so on.[8 21 22] Nevertheless, pericardial fluid concentrations of tAM and mAM in group R were significantly increased over those in group N. In addition, we found that tAM and mAM in pericardial fluid correlated with indicators of left ventricular function, while plasma tAM and mAM did not. Overall, our results clearly show that pericardial fluid adrenomedullin reflects cardiac function more accurately than plasma adrenomedullin.

Our study also showed that the concentration of pericardial fluid mAM was significantly higher than plasma mAM in both groups. The ratio of the mAM concentration to the tAM concentration in pericardial fluid was twice as high as in plasma. The significantly higher concentration of mAM in pericardial fluid—not only in absolute terms but also in relation to the ratio with tAM levels—leads to the following considerations. Firstly, the heart may actively secrete mAM, which is compatible with a recent study that plasma mAM concentrations were significantly increased in the coronary sinus.[29] Secondly, pericardial fluid, epicardium, pericardium, or the heart itself may have a greater capacity for enzymatic amidation than plasma, the amidation process being necessary for conversion of iAM to mAM.[4]

Another concept is related—that mAM functions in the heart by an autocrine or paracrine mechanism. If it works in this way, it is likely that mAM produced in the heart binds and acts in situ, and that very little is released into the blood circulation.[5] There is increasing evidence from experimental models that adrenomedullin has a wide range of autocrine or paracrine functions in various organs, including inhibition of proliferation, differentiation, migration, or apoptosis of the cells.[8] Concerning the heart, adrenomedullin has been shown in vitro to have direct positive inotropic effects on cardiomyocytes[9] and inhibitory effects[10 11] on protein synthesis in cardiac myocytes and fibroblasts. In addition, mechanical stretching has recently been reported to stimulate mRNA expression and peptide secretion of adrenomedullin in cultured rat cardiomyocytes, which strongly supports the view that adrenomedullin participates in mechanisms opposing the cardiac hypertrophy induced by pressure and volume overload of the heart.[12]

While the cardioprotective functions of adrenomedullin as a local mediator have already been implicated in in vivo studies,[13 14] few have addressed human hearts. Although one report showed an association between plasma adrenomedullin concentrations and ventricular hypertrophy in patients with essential hypertension, this focused on the functions of adrenomedullin in relation to hypertension, without mentioning its autocrine or paracrine activities in the heart.[30] The finding that pericardial fluid mAM was better correlated with left ventricular haemodynamic variables than plasma mAM, and that the concentrations of mAM were higher in the pericardial fluid than in plasma, both in absolute terms and as a ratio, may well reflect the autocrine or paracrine functions of adrenomedullin in the heart in patients with cardiac remodelling. The stability of the mAM/tAM ratios in pericardial fluid in our study supports this speculation as well, as it suggests the participation of a particular dominant factor causing adrenomedullin secretion into the pericardial fluid, irrespective of other secretion triggers.

Apart from the factors directly related to left ventricular remodelling, there were no significant differences in the clinical background between our two patient groups except for the numbers of patients with a history of myocardial infarction (table 1). Although this could have affected our results if old myocardial infarction gave rise not only to cardiac remodelling but also to current myocardial ischaemia, there were no

differences in adrenomedullin concentrations between the patients with and without apparent ischaemia (unstable angina) in group R (data not shown). While it has been reported in various studies that tissue hypoxygenation induces the production of adrenomedullin,[31 32] mechanical stretching seemed to be a more potent stimulator of adrenomedullin secretion than hypoxia.

Limitations

We did not investigate the origins of tAM or mAM directly. It is possible that different clearance mechanisms of adrenomedullin in plasma and pericardial fluid were implicated in the higher pericardial fluid concentrations. Although the lung has been reported to be a major clearance site of circulating tAM and mAM,[29 33] the precise metabolic pathways of adrenomedullin remain to be elucidated.

Conclusions

Patients with cardiac remodelling had significantly higher concentrations of mAM in pericardial fluid than in plasma, both in absolute terms and as a ratio to tAM concentrations. Pericardial fluid mAM concentration was correlated with left ventricular haemodynamic variables, while plasma concentration was not. The biochemical characteristics of mAM and pericardial fluid suggest that adrenomedullin has autocrine or paracrine functions opposing cardiac remodelling in patients with left ventricular dysfunction.

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