PREVIOUS REPORTS DEMONSTRATED that plasma levels of adrenomedullin (AM) are increased in patients with heart failure (12, 16, 20, 23) and ventricular hypertrophy (4). Recent reports suggest that, other than its effects on vascular tone and natriuresis, physiological concentrations of AM may influence myocardial inotropy (7, 27). Several groups demonstrated the importance of AM as an auto- and paracrine mechanism for modulation of cellular growth and development (3, 8, 21, 28). As a strong nitric oxide-liberating peptide in the vascular system, systemic and coronary circulation is regulated by AM (5). These results indicate that AM might be involved in the pathophysiology of cardiac hypertrophy and heart failure. Where circulating AM originates from is still debated, but recent data suggest that cardiac AM expression contributes to elevated serum levels in patients with heart failure (9, 29). Under physiological conditions, atrial levels of AM are among tissues of highest expression in the body. High levels of atrial expression (21), its colocalization with the expression of atrial natriuretic peptide, and almost identical physiological actions of AM and atrial natriuretic factor (ANF) (21) led to the hypothesis that, in addition to its local auto- and paracrine effects, AM may also act as a humoral factor of cardiorenal homeostasis.

In the present study, gene expression of AM in atrial and ventricular myocardium at two stages of the development of chronic heart failure in spontaneously hypertensive rats (SHR) and idiopathic human heart failure were investigated. Therefore, hearts of SHR with chronic ligation of the left coronary artery as a well-characterized model of congestive heart failure (CHF) and myocardium of explanted human hearts with end-stage heart failure due to idiopathic dilated cardiomyopathy (DCM) were investigated. The expression levels of AM were compared with the expression of ANF, a sensitive marker of cardiac hypertrophy and failure. Furthermore, we performed contraction studies on isolated rat papillary muscles of sham-operated (sham) controls or to investigate functional effects of AM in normal and failing myocardium.

METHODS

Postinfarction animal model. All experiments were performed in accordance with the German animal protection law. Male SHR (age = 16 wk; Haarlan Winkelman) were anesthetized, and a thoracotomy was performed as described (17). The left coronary artery was ligated 2 mm beneath its origin. The sham operation consisted of the identical procedure except for the coronary ligation. Animals were kept for 2 wk under standard conditions. Then, infarct size was measured by NMR technique. Only rats with infarct sizes of 20–40% of left ventricular mass were used. Subsequently,
rats were kept for another 16 wk under standard conditions for development of heart failure. At the time of inclusion into the study, rats did not show clinical signs of cardiac decompensation.

**Human myocardium.** Experiments were performed on failing myocardium from eight patients undergoing heart transplantation due to idiopathic DCM (New York Heart Association class IV; mean age = 50.7 ± 10 yr). Patients with ischemic cardiomyopathy (coronary angiography) were excluded. Medical therapy of the patients consisted of digitalis, diuretics, nitrates, and angiotensin I-converting enzyme inhibitors. None of the patients had received catecholamines. All patients gave written, informed consent before operation. Nonfailing (mean age = 44 ± 12.9 yr) myocardiums from 10 organ donors, whose hearts could not be used for transplantation due to technical reasons or blood group incompatibilities, were used as controls. The major source of donor hearts was from persons dying of spontaneous intracerebral or subarachnoidal bleeding. Patient histories of donors of nonfailing hearts as well as two-dimensional echocardiography revealed no evidence of heart disease. All explanted hearts were perfused with ice-cold cardioplegic solution and kept on ice until they were transported within 1 h to the laboratory where myocardial samples were snap frozen in liquid nitrogen.

**Isolation of total RNA and Northern blot analysis.** Total RNA was isolated from myocardial tissue and was analyzed as described previously (1). In brief, 25 μg of total RNA were separated by gel electrophoresis, blotted on nylon membranes, and fixed by ultraviolet cross-linking. The blots were hybridized with random-primed 32P-radiolabeled cDNA probe for human AM, human ANF, and glycerolaldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The signals on the autoradiographs were quantified by densitometry. GAPDH was used as an internal control to normalize for differences in loading of RNA.

**Reverse transcription-polymerase chain reaction.** For the determination of rat mRNAs, semiquantitative PCRs were developed. Rat ANF mRNA was amplified by using the following primers and conditions: 5’-GGTTAGGATGACAGGATGGAG-3’ (sense), 5’-CGTGTAGTGGAAAGACAGGAAG-3’ (antisense), at 94°C for 30 s, 55°C for 30 s, 72°C for 45 s, and 23 cycles, leading to a 198-base pair (bp) product. Rat AM was amplified by using the following primers and conditions: 5’-TTACAGACAAAAGACAGGAAG-3’ (sense), 5’-TTACACACACACACACACAC-3’ (antisense), at 94°C for 30 s, 56°C for 30 s, 72°C for 60 s, and 28 cycles, leading to a 736 bp product. For quantification of results, GAPDH was amplified from the same cDNA as a housekeeping gene with the following primers and conditions: 5’-ACCACACTCCATGCCATCAG-3’ (sense), 5’-TCCACCACCTGGTTGGTCTGA-3’ (antisense), at 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, and 23 cycles, leading to a 450-bp product. cDNAs were densitometrically evaluated after gel electrophoresis.

**Isolated papillary muscle studies.** Contraction studies with freshly isolated papillary muscle were performed as published previously (25). In brief, isometric force of contraction was recorded. Twelve weeks after the coronary ligation or sham operation, the hearts were excised and left ventricular noninfarcted papillary muscles were dissected free. The muscles were suspended in organ baths and were electrically stimulated (frequency = 1 Hz, impulse duration = 5 ms, voltage 10–20% above threshold). Before stimulation procedures, baseline data had been recorded after a 30-min equilibration period. Rat AM was purchased from American Peptide (Sunnyvale, CA).

**Statistical analysis.** All data are described as means ± SE. Statistical significance was estimated by using the Student’s t-test for unpaired observations or one-way ANOVA with Fisher’s least-significant difference as the post hoc test. A P value of <0.05 was considered significant.

**RESULTS**

**Characterization of the animal model of compensated CHF.** SHR with chronic myocardial infarction display clinical signs (peripheral edema, pleural effusion) of CHF depending on the size of myocardial infarction and length of the postinfarction time interval. One aim of this study was to investigate developing heart failure in this rat model. Therefore, animals with a comparably small loss of ventricular myocardium (20–40%) were chosen. Our study animals with chronic ligation of the left coronary artery did not display signs of overt heart failure. The lung water content was 1.53 ± 0.19 vs. 1.49 ± 0.11 g in control animals (not significant). Furthermore, body weight and liver wet weight were similar in CHF and sham animals, whereas hearts showed typical fibrous scars due to myocardial infarction (Fig. 1). Yet after a prolonged life span after myocardial infarction, animals with a comparably small loss of ventricular myocardium also develop CHF.

**Expression of rat AM compared with rat ANF.** First, the influence of CHF on mRNA expression of the two hormones of the cardiorenal homeostasis (ANF and AM) was investigated. Examination of noninfarcted left ventricular myocardium revealed increased ANF message levels in CHF compared with sham rats and as assessed by semiquantitative PCR (Fig. 2A; P < 0.05). Increased myocardial ANF expression is a widely accepted early sign of myocardial remodeling of the surviving tissue. Unlike ANF, AM message levels were not significantly changed (Fig. 2B). Yet AM message levels of left ventricular myocardium were increased compared with the right ventricle, which may indicate activation of the AM hormonal system. In sham ani-

![Fig. 1. Excised heart of a spontaneously hypertensive rat (SHR) 12 wk after ligation of the left coronary artery and clinical signs of chronic heart failure. Scar of the free left ventricular wall 3 mo after infarction is due to ligation of the left coronary artery (and infarction of two-thirds of left ventricular wall myocardium).](http://jap.physiology.org/)
mals, the overall expression of AM in ventricular myocardium appeared slightly lower than in atrial tissue (not significant).

**Myocardial force of contraction.** AM may modulate cardiac function as an autocrine or paracrine factor. So far, conflicting data exist on its importance for myocardial inotropy (see DISCUSSION). Therefore, experiments on rat papillary muscles were performed to elucidate the influence of AM on myocardial contractility. Species-specific AM was added to organ baths with freshly excised and electrically paced papillary muscle preparations. Exemplary original mechanograms show that AM did not influence myocardial inotropy even at supraphysiological doses (1.0 μmol/l; Fig. 3). To elucidate potential negative inotropic influences via inhibitory G proteins of the β-adrenergic adenyl cyclase-cAMP cascade, papillary muscle preparations were prestimulated with 0.03 μmol/l isoprenaline. This concentration caused a half-maximal increase in force of contraction. In these experiments, AM did not show anti-adrenergic effects on myocardium. Control experiments with freshly excised aortic segments of these rats showed the vasodilating effect of AM (not shown). All experiments were performed on preparations of several sham-operated animals and rats with CHF (n = 4 each). AM was added to the organ baths in cumulative doses from 0.001 to 1.0 μmol/l (not shown) and single doses (1.0 μmol/l; Fig. 3).

**Expression of human AM compared with human ANF.** Further studies were performed on human cardiac tissue. To elucidate the role of AM in human heart failure, AM and ANF message levels were investigated in myocardium of all four heart chambers in explanted hearts by Northern analyses. In nondiseased hearts, atrial AM message levels were approximately threefold higher than ventricular message levels, whereas atrial ANF message levels were ~15-fold higher compared with ventricular myocardium (not shown). In DCM, AM expression was significantly increased in the right and left ventricles (Fig. 4, bottom, P < 0.05) compared with myocardium of nondiseased hearts. In parallel, ventricular ANF expression was increased less robustly (P < 0.01). In right and left atrial myocardium, AM and ANF message levels were similar (Fig. 4, top).

**DISCUSSION**

Our data on AM mRNA expression support results from recent investigations, which showed increased AM immunoreactivity in atrial myocardium compared with ventricles (10, 11). Yet, in our hands, Northern blotting did not lead to signals in adult rat myocardium of SHR, which suggests low expression levels compared with human myocardium or other rat strains (29). In the state of chronic ligation of the left coronary artery in this rat model, ANF expression was increased as a marker of myocardial remodeling. Examination of left ventricular AM expression could not disclose an in-
crease in CHF vs. sham. Compared with right ventricles, AM message levels were significantly increased in left ventricular myocardium of CHF. In human myocardium, AM expression was increased similarly in left and right ventricles of patients with DCM compared with nonfailing myocardium. The increased levels of AM expression were more robust than ANF-mRNA expression, of which it is well known that the ventricular expression is highly variable in human heart failure. Therefore, our data support the study by Jougasaki et al. (12), which showed increased AM immunoreactivity in myocardium of patients with CHF. Rat AM mRNA-expression studies suggest that there are species differences as well as differences in each model of cardiomyopathy. In addition, there appears to be a great variability in the correlation of cardiac AM protein and mRNA expression; e.g., in healthy male Wistar rat myocardium (6, 29), AM tissue concentrations are 10–20 times higher in atrial myocardium than in ventricular myocardium, whereas mRNA expression appears to be increased by only 20%. The latter data are similar to our results from sham SHR. In some models of volume or pressure-overload cardiac myopathy, ventricular AM mRNA expression was found to be increased (26, 29), whereas in hypertensive TGR(mREN-2)27 rats, ventricular AM levels were increased without enhanced mRNA expression (24). Also, in a surgical rat model of pressure overload, no change of AM, despite a marked increase of ANF expression, was seen (13). Therefore, AM appears to be an independent marker of specific cardiac pathophysologies compared with other humoral factors like ANF. As our results demonstrate, ventricular AM expression is robustly enhanced in human end-stage heart failure due to DCM.

In the cardiovascular system, AM acts as a regulatory peptide in cellular growth and differentiation, a modulator of hormone secretion, and a hypotensive regulator.
peptide via a nitric oxide-dependent pathway (3). AM acts most likely by interaction with G-protein-coupled AM receptor(s) (AR). Few data exist about the distribution and their function in certain diseases like heart failure. ARs belong to the family of AM or calcitonin gene-related peptide (CGRP) receptors with distinct binding characteristics for their ligands. The first of these receptors was cloned in 1993 by Harrison et al. (2) and characterized as an orphan receptor. Studies by Kapas et al. (14) suggested selective binding with a high affinity for AM by this putative AR, but recent studies failed to prove its role as a functional AM receptor (15). McLatchie et al. (18) showed that coexpression of receptor-modifying proteins 1, 2, and 3 (RAMP1, -2, and -3, respectively) with the calcitonin receptor-like receptor (CRLR) modulate the specificity of AM and CGRP receptors to the ligands CGRP or AM (19). Coexpression of RAMP2 with the CRLR receptor leads to glycosylation determining its specificity for AM. Recently, Pio et al. (22) found, in vitro experiments, that a serum binding protein for AM (complement factor H) enhances the activity of AM at its receptor. These insights from in vitro experiments increase the understanding of the physiological cascade of AM. However, no studies on these proteins and interactions with the CRLR in heart failure are yet available.

The role of AM in the modulation of cardiac contractility discussed is contradictory. Szokodi et al. (27) reported that, in Langendorff-working heart preparations, AM exhibits a positive inotropic effect via a Ca²⁺-dependent mechanism. In contrast, AM led to a negative inotropic effect in isolated rabbit myocytes. This was mediated by a nitric oxide-dependent mechanism (7). In our electrically paced rat papillary preparations, neither positive nor negative inotropic effects could be demonstrated even at supraphysiological concentrations. Furthermore, AM showed no antidiurenergic effect after prestimulation with isoprenaline, which could have disclosed the involvement of inhibitory G proteins. These findings put forward the following suggestion: AM may act mainly as a regulator of vascular tone and thereby influence myocardial perfusion. As a secondary effect, contractility may be affected. Usually, freshly excised and intact papillary muscles as a multicellular preparation are reliable in predicting modulatory influences on myocardial inotropy, e.g., compared with single cell preparations, which need enzymatic dispersion during the isolation procedure. Therefore, we are confident that AM does not significantly influence myocardial contractility in SHR.

In conclusion, in human DCM, AM expression is upregulated parallel to ANF. AM expression appears to be not involved in SHR postinfarction myocardial remodeling, in contrast with ANF. In cardiac pathophysiology, AM may function as a locally acting factor and mediator in cardio-renal homeostasis without directly influencing myocardial contractility.

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