Upregulation of adrenomedullin and its receptor components during cardiomyocyte hypertrophy induced by chronic inhibition of nitric oxide synthesis in rats

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Adrenomedullin (AM) possesses vasodilator (30) and natriuretic (41) effects. AM was first isolated from adrenal medulla (41) but also is found in plasma and in the heart (29, 33, 56, 62). Increased plasma levels of AM have been detected in hypertensive patients with LVH (56), myocardial infarction (29), and heart failure (33, 62); levels are greater in hypertensive patients with LVH than in hypertensive patients without underlying LVH (56). Increased ventricular levels of AM correlate with increased LV weight-to-body weight ratio in pressure overload hypertrophy in rats; infusion of AM reduced LV weight-to-body weight.

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Cardiomyocytes express the precursor peptide mRNA and secrete the mature AM peptide (17, 29), and the mean of four consecutive blood pressure readings was obtained for each animal at weekly intervals. Body weight was recorded weekly and water consumption daily.

**Cardiomyocyte isolation.** After deep anesthesia of the rats with isoflurane (Abbott Laboratories, Cambridge, UK), the hearts were rapidly excised, placed in ice-cold saline, and weighed. Excised hearts were cannulated through the ascending aorta, and preparations of cardiomyocytes were isolated from the left and right ventricles, respectively, by enzymatic digestion (0.4 mg/ml collagenase) using Langendorff perfusion (8). After purification, cells were utilized immediately for extraction of RNA (RT-PCR protocols) or, for assessment of protein turnover, suspended at 1.5 × 10^6 viable cardiomyocytes per milliliter in a “creatinine-carnitine-taurine” (CCT) medium, which consisted of modified glucose-free medium supplemented with Earle’s salts (GIBCO, Paisley, UK), HEPES (15 mM), creatinine (5 mM), l-carnitine (2 mM), taurine (5 mM), ascorbic acid (100 μM), penicillin (100 IU/ml), and streptomycin (100 μg/ml). The medium also was supplemented with cytosine β-D arabinofuranoside (10 μM) to prevent growth of nonmyocytes (Sigma Chemical, Poole, UK).

**Assessment of cardiomyocyte protein synthesis.** Petri dishes (35-mm diameter) were preincubated for 2 h with fetal calf serum (4% vol/vol) in CCT medium. Aliquots of cell suspension (1 ml) were pipetted gently onto petri dishes, and after 1 h, viable cardiomyocytes became attached to the surface of the dish. The dishes were then washed with fresh CCT medium to remove nonattached cells, and cell debris and the attached cells were exposed for 24 h to l-[U-14C]phenylalanine (0.1 μCi/ml culture medium; Amersham Pharmacia Biotech UK). Incorporation of radioactivity into the acid-insoluble cell fraction was determined under basal conditions and in the presence of a hypertrophic stimulus, PMA (100 nM) ± AM (10^{-12}–10^{-7} M) (8). The attached cells were then washed with an aliquot (1 ml) of ice-cold PBS before the addition of an aliquot (1 ml) of ice-cold trichloroacetic acid (10% wt/vol). After storage overnight at 4°C, the acid containing the intracellular precursor pool was removed from the dishes and the attached cells were washed with an aliquot (1 ml) of PBS. The precipitate remaining on the culture dishes was dissolved in an aliquot (1 ml) of NaOH (0.1 M)-sodium dodecyl sulfate (0.01% wt/vol) by incubation at 37°C. In these samples, concentration of DNA was determined using a spectrophotometric method in which bisbenzamidine dye was incorporated into DNA (7), the concentration of

**METHODS**

**Experimental model.** The study was approved by the Department of Health, Social Services, and Public Safety (Northern Ireland) under the terms of the Animals (Scientific Procedures) Act 1986. Eight-week-old male Sprague-Dawley rats were assigned to receive L-NAME (20 or 50 mg·kg⁻¹·day⁻¹ in drinking water) or drinking water only (age-matched control) for 4 or 8 wk and were maintained at the Laboratory Service Unit, The Queens University of Belfast, before death at 12 or 16 wk of age. Systolic blood pressure (SBP) was determined by tail-cuff sphygmomanometer (Harvard Instruments) (7), and the mean of four consecutive blood pressure readings was recorded weekly and water consumption daily.

### Table 1. Primer sequences

<table>
<thead>
<tr>
<th></th>
<th>Forward</th>
<th>Backward</th>
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<tr>
<td>AM</td>
<td>CATTGAAAGCTGCCGGGAGTA</td>
<td>GTGCGGAAGCTCATCTGATTCC</td>
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<tr>
<td>BC061775</td>
<td>21 bp (739-759)</td>
<td>21 bp (776-796)</td>
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<td>RAMP-1</td>
<td>AGCACCAGGGAGCTCATG</td>
<td>GACACCAACGGGAGCTCATG</td>
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<tr>
<td>AB042887</td>
<td>21 bp (268-288)</td>
<td>19 bp (315-333)</td>
</tr>
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<td>TGCGGAAGCTTGGCGAACGTG</td>
<td>AGGCAAATCGGCGGTGCTGT</td>
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<tr>
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<td>20 bp (315-334)</td>
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<td>TGCTCCACATCCGTTGGAGTT</td>
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<td>GCTGATAGGCTCCGAGAAGATGCTGAG</td>
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<td>28 bp (1102-1129)</td>
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<td>TGCCGCTCTTGTAGTTGGCAAG</td>
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<td>M64711</td>
<td>21 bp (347-367)</td>
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<td>GGTCTGATACTCCTGGT</td>
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<td>BC061974</td>
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<td>21 bp (247-262)</td>
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<td>GGACACCTCAGTCTTGGT</td>
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<td>19 bp (300-318)</td>
<td>20 bp (333-352)</td>
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<td>M25297</td>
<td>19 bp (71-89)</td>
<td>20 bp (102-121)</td>
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</table>

Accession numbers are taken from the European Molecular Biology Laboratory (EMBL) database, which is part of the International Nucleotide Sequence Database Collaboration. AM, adrenomedullin; RAMP, receptor activity-modifying protein; CRLR, calcitonin receptor-like receptor; ET-1, endothelin-1; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.
protein was determined using the Lowry colorimetric method, and the radioactivity was counted. The ratio of \( \text{U-}^{14}\text{C phenylalanine incorporated to DNA per culture served as a measure of de novo synthesis of protein, and the ratio of protein to DNA per culture served as a measure of cellular protein mass.} \\

*Real-time PCR.* Reported sequences for each gene (Table 1) were used to design, with Primer Express software (PE Applied Biosystems), rat-specific primers adapted to RT-PCR conditions, which were synthesized by Invitrogen. RT-PCR was performed using the following cycle parameters: 10 min at 95°C, followed by 40 cycles of 15 s at 95°C and 1 min at 60°C, using the ABI Prism sequence detector (PE Applied Biosystems). For each gene, RT-PCR was conducted in duplicate using Absolute QPCR SYBR green ROX (Abgene) in a 2:1 reaction. To ensure the quality of measurements, we included both negative and positive controls in each plate. Analysis was performed using ABI 7000 Prism software. The conversion between Ct and relative gene expression level is performed using the Ct value \( \text{Ct(gene of interest)} \text{calibrator} \) by using the control group as a calibrator for comparison of every unknown sample gene expression level. The conversion between Ct and relative gene expression level is \( 2^{-\text{Ct(sample)} - \text{Ct(calibrator)}} \) by using the control group as a calibrator for comparison of every unknown sample gene expression level. \\

*Data analysis.* Data are expressed as means \( \pm \text{SE, where n denotes either the number of rats in which SBP or heart weight-to-body weight ratio was measured or the number of heart cell preparations used to analyze gene expression or protein synthesis. Statistical analyses were performed using analysis of variance to detect significant differences for between-group or within-group effects, and post hoc comparisons were made using Bonferroni’s method or an unpaired Student’s \text{t}-test as appropriate.} \\

**RESULTS** \\

*Systolic blood pressure.* SBP was greater \( (P < 0.05) \) in rats treated with \( \text{L-NAME} \) 20 and 50 mg \( \text{kg}^{-1} \text{day}^{-1} \) at 10 wk of age onwards (i.e., after 2 wk of treatment with drug) relative to age-matched control values. Maximum increase was observed at 14 wk of age and was 34.2 mmHg and 104.9 mmHg greater, respectively, than age-matched control values (Fig. 1A). \\

*Heart weight-to-body weight ratio.* Heart weight-to-body weight ratio was not different between control animals at 12 and 16 wk of age and was not altered by treatment of 8-wk-old animals with 20 mg \( \text{kg}^{-1} \text{day}^{-1} \) \( \text{L-NAME} \) for 8 wk or with 50 mg \( \text{kg}^{-1} \text{day}^{-1} \) \( \text{L-NAME} \) for 4 wk (Fig. 1B). Treatment with 50 mg \( \text{kg}^{-1} \text{day}^{-1} \) \( \text{L-NAME} \) for 8 wk did increase \( (P < 0.05) \) the ratio by 24.1% relative to the age-matched control value; however, this was accounted for largely by a reduction of 15.9% \( (P < 0.05) \) in body weight (animals treated with the higher dose of \( \text{L-NAME} \) did not gain weight during the last 1–2 wk of treatment, and the majority displayed some weight loss). Heart weight did not vary significantly with age or treatment (data not shown). \\

**Hypertrophic parameters in ventricular cardiomyocytes.** The protein mass and extent of basal synthesis of new protein each decreased between 12 and 16 wk of age \( (P < 0.05) \) in both LV and RV cardiomyocytes obtained from control rats. Relative to age-matched control values, the protein mass of cells treated with \( \text{L-NAME} \) was not different at 12 wk, whereas an increase was observed at 16 wk; the maximum increases upon treatment with 50 mg \( \text{kg}^{-1} \text{day}^{-1} \) \( \text{L-NAME} \) were 22.4% greater than control value in LV and 59.0% greater than control value in RV, but these increases were not statistically significant (Fig. 2, A and C). Basal protein synthesis (Fig. 2, B and D) was increased \( (P < 0.05) \) at 16 wk of age in cells treated with 20 and 50 mg \( \text{kg}^{-1} \text{day}^{-1} \) \( \text{L-NAME} \) by 93 and 66%, respectively, relative to the age-matched control value in LV car-

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Fig. 1. A: temporal dependence of the effect of chronic administration of \( \text{N}^\text{ω}-\text{nitro-L-arginine methyl ester (L-NAME; 20 and 50 mg} \text{kg}^{-1} \text{day}^{-1}) \) to 8-wk-old rats on systolic blood pressure (BP). Data are means \( \pm \text{SE of 4–12 (50 mg} \text{kg}^{-1} \text{day}^{-1}) \text{, 5–7 (20 mg} \text{kg}^{-1} \text{day}^{-1}) \text{, and 6 control rats. *P < 0.05, significant variation from paired control response.} \\

B: effect of chronic administration of \( \text{L-NAME} \) (20 and 50 mg \( \text{kg}^{-1} \text{day}^{-1}) \) to 8-wk-old rats on heart weight-to-body weight ratio. Data are means \( \pm \text{SE of 6–13 rats. *P < 0.05, significant difference from age-matched control response.} \\

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diomyocytes and by 137 and 137%, respectively, relative to the age-matched control value in RV cardiomyocytes.

The hypertrophic stimulus, PMA (100 nM), increased protein synthesis above basal values ($P < 0.05$) in all experimental groups; i.e., both RV and LV cells from control and L-NAME-treated rats. The response to PMA tended to be greater in L-NAME (50 mg·kg$^{-1}$·day$^{-1}$)-treated LV cells at 16 wk (40.1 ± 8.3% above basal values, $n = 13$) relative to control LV cells (28.5 ± 3.1%, $n = 14$), but differences were not statistically significant; in contrast, the PMA response was not increased in RV cells (26.9 ± 7.0%, $n = 12$ above basal values) relative to control values (34.4 ± 4.1%, $n = 16$).

Expression of skeletal α-actin (3.3- and 2.6-fold), ET-1 (2.7- and 3.9-fold), and brain natriuretic peptide (BNP; 2.9- and 3.1-fold) mRNAs by LV cardiomyocytes was increased ($P < 0.05$) relative to that of age-matched cells at 16 wk by the lower and higher dose of L-NAME, respectively (Fig. 3, A, B, and C). Expression of ANP mRNA was not affected by treatment with 20 and 50 mg·kg$^{-1}$·day$^{-1}$ L-NAME but was increased 17.9-fold in cells obtained from rats treated with 50 mg·kg$^{-1}$·day$^{-1}$ L-NAME (Fig. 3C). There was a positive correlation between expression of hypertrophic genes and anatomical hypertrophy, as evidenced by heart weight-to-body weight ratio (Fig. 4). Gene expression was not affected in RV cardiomyocytes by treatment with L-NAME (data not shown).

Expression of adrenomedullin and receptor components. Expression of AM mRNA was increased ($P < 0.05$) in a concentration-dependent manner in LV cardiomyocytes from rats treated with 20 and 50 mg·kg$^{-1}$·day$^{-1}$ L-NAME relative to cells from age-matched control rats at 16 wk (Fig. 3E) but was not changed in RV cells (data not shown). There was a positive correlation between expression of AM mRNA in LV cardiomyocytes and anatomical hypertrophy, as evidenced by heart weight-to-body weight ratio (Fig. 4E). In LV cardiomyocytes from animals treated with 20 mg·kg$^{-1}$·day$^{-1}$ L-NAME, expression of AM’s receptor components was also determined: expression of RAMP2 and RAMP3 mRNAs was increased ($P < 0.05$); CRLR mRNA and RAMP1 mRNA levels also were elevated but were not significantly greater than in cells from control animals (Fig. 5).
Antihypertrophic effect of adrenomedullin in vitro. Protein mass and protein synthesis, as evidenced by [14C]phenylalanine incorporation of ventricular cardiomyocytes isolated from the hearts of control rats at 12 wk of age and incubated (24 h) in the presence of AM (10⁻¹²–10⁻⁷ M), were not different from basal values; in contrast, PMA (10⁻⁷ M), employed as a positive control, increased (P < 0.05) protein mass (25.6%) and protein synthesis (35.3%). AM (10⁻¹²–10⁻⁷ M) attenuated PMA-stimulated increases in protein mass and synthesis concentration dependently; at 10⁻⁷ M, the increase in protein mass was abolished and protein synthesis was attenuated by 75.5% (P < 0.05) (Fig. 6).

Fig. 3. Effect of chronic administration of L-NAME (20 and 50 mg·kg⁻¹·day⁻¹) to 8-wk-old rats for 8 wk on expression of skeletal (sk) α-actin (A), endothelin (ET)-1 (B), atrial natriuretic peptide (ANP; C), brain natriuretic peptide (BNP; D), and adrenomedullin (AM) mRNA (E) in LV cardiomyocytes. Data are expressed relative to GAPDH mRNA levels and are means ± SE of 6–9 heart cell preparations. *P < 0.05; **P < 0.01; ***P < 0.005, significant variation from control response.
DISCUSSION

Cardiac hypertrophy. Heart weight-to-body weight ratio was increased, but only at the higher dose of L-NAME, to a greater extent than changes reported in another model of pressure overload, the spontaneously hypertensive rat (SHR), despite similar changes in SBP (7). However, unlike the SHR, heart weight was not increased in the L-NAME model, in agreement with the findings of Pechanova and Bernatova (36) but, in contrast to some other reports (10, 20, 38, 47, 48), was reduced. Indeed, reduction in body weight is frequently observed after administration of moderate to high doses of L-NAME (5, 20, 46, 47, 48). Reduced food and water intake

Fig. 4. Relationship between heart weight-to-body weight ratio and expression of skeletal $\alpha$-actin (A), ET-1 (B), ANP (C), BNP (D), and AM mRNA (E) in LV cardiomyocytes. Data were pooled from values obtained from rats to which L-NAME (20 and 50 mg·kg$^{-1}$·day$^{-1}$) was administered for 8 wk from 8 wk of age and from untreated age-matched control group.
following prolonged administration of the higher dose, combined with the observation of increased morbidity and mortality, is also in agreement with previous reports (13, 35, 58) and indicates that a dosing schedule intermediate between low (20 mg·kg\(^{-1}\)·day\(^{-1}\)) and moderate (50 mg·kg\(^{-1}\)·day\(^{-1}\)) may be better tolerated and preferable for establishing a chronic stable model of myocardial remodeling in NO-deficient hypertension. It is probable that demonstration of increased heart weight-to-body weight ratio in the present study is truly indicative of anatomical hypertrophy, because heart weight is maintained in the presence of a significant decrease in the weight of other constituents of the body. Furthermore, there are a number of reports of increased LV weight (47, 48), thickness of myocardial fibers (54), and LV area (1, 31). It is unlikely that an increase in LV weight specifically would have been offset by no change or decrease in RV weight, although this has been observed in some studies (9, 38, 53, 57), because Hampel et al. (16) reported modest changes in both chambers and similar elevation in SBP and decreased body weight at doses of L-NAME equivalent to those employed in our study, and in agreement with our observed trend in both LV and RV cardiomyocytes toward increased protein mass, which also provides further evidence for the existence of anatomical hypertrophy. Changes at organ level reflect cardiomyocyte hypertrophy, proliferation of nonmyocytes, and deposition of extracellular matrix (51). Local NOS deficiency, independent of increased blood pressure, may underlie some of the changes associated with myocardial fibrosis and remodeling of nonmyocytes (53). A number of studies have examined the histology of cardiomyocytes within tissue sections (24, 47, 48, 58), but to our knowledge there has been only one other study utilizing cardiomyocytes isolated from L-NAME-treated animals (5). Increased cardiomyocyte cross-sectional area has been reported after chronic administration of high (24, 47, 48, 58) but not moderate (5) doses of L-NAME. The inability of the blood pressure-lowering agent hydralazine to attenuate these increases indicates that they are likely to be accounted for by NO deficiency within the myocardium, rather than by arterial hypertension; LV NOS activity is attenuated markedly after chronic administration of L-NAME (9, 38, 53, 64). Decreased NO levels may compromise local blood flow within the myocardium, thereby promoting ischemia and leading to cardiomyocyte hypertrophy and necrosis (12, 13).

Cardiomyocyte protein mass. Cardiomyocyte protein synthesis was stimulated similarly by administration of low and moderate doses of L-NAME for 8 wk. Although there was a trend toward increased protein mass at the higher dose, increases were less apparent than those observed in SHR cardiomyocytes under similar pressure loading (7). Bartunek et al. (5) proposed that L-NAME might suppress increased mass in the LV because of a negative metabolic effect involving reduced amino acid delivery and utilization and altered ornithine metabolism. The present finding of enhanced cardiomyocyte protein synthesis following chronic administration of low to moderate doses of L-NAME does not support this conclusion but is compatible with the observation that NO itself may exert direct inhibitory effects on cell growth and proliferation (5, 22, 49). Although enhanced protein synthesis is associated with the

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**Fig. 5.** Effect of chronic administration of L-NAME (20 mg·kg\(^{-1}\)·day\(^{-1}\)) to 8-wk-old rats for 8 wk on expression of calcitonin receptor-like receptor (CRLR; A), receptor activity-modifying protein 1 (RAMP1; B), RAMP2 (C), and RAMP3 mRNA (D) in LV cardiomyocytes. Data are expressed relative to GAPDH mRNA levels and are means ± SE of 6 heart cell preparations. *P < 0.05; ***P < 0.005, significant variation from control response.
hypertrophic growth response to pressure overload, similar changes were observed in both ventricles, and despite a greater increase in SBP at the higher dose, protein synthesis did not increase further, indicating that in the L-NAME model these changes are likely to reflect NO deficiency, rather than pressure overload. This conclusion is supported by the studies of Pech-anova and colleagues (9, 10, 38), who reported that although increased protein synthesis was evident in sections of LV tissue after administration of moderate doses of L-NAME, this effect also occurred in other tissues, notably aorta, brain, and kidney, and in the myocardium was associated, at least in part, with proliferation of nonmyocytes and enhanced synthesis predominantly of extracellular matrix proteins and metabolic proteins, such as mitochondrial and cytoplasmic enzymes that are required to meet the increased metabolic demand, rather than contractile proteins. Such changes were not attenuated by blood pressure reduction per se (45). Hampl et al. (16) demonstrated that chronic L-NAME intake, while causing remarkable hypertension in the systemic vascular bed, does not enhance mean pulmonary arterial pressure or alter pulmonary vascular morphology, indicating that pressure overload cannot account for the increased protein mass of RV cardiomyocytes. The trend toward increased activity of the direct activator of PKC, PMA, when applied to LV cardiomyocytes in vitro may reflect enhanced activity of myocardial S6 kinase and ERK signaling cascades, which are known to be stimulated by PKC and are upregulated after chronic administration of high doses of L-NAME (45, 53).

**Hypertrophic gene expression.** Reinduction of skeletal α-actin within cardiomyocytes from the LV only, even in the absence of cardiac mass increase, after administration of L-NAME is in agreement with previous findings in whole tissue (5, 14, 57) in which variable levels of hypertrophy were reported. Induction of skeletal-α-actin also occurs in SHR.
before elevation in cardiac mass after the onset of hypertension (7) and has implications for cardiac contractility. Together, these data indicate that reinduction of this structural gene occurs in response to pressure overload on the LV but is not observed in the unloaded RV (16). Similarly, upregulation of the expression of the natriuretic peptide BNP and the hypertrophic growth factor ET-1 might also represent an adaptive response to increased mechanical load in the affected ventricle. A linear relationship was noted between the levels of expression of these genes in LV cardiomyocytes and the extent of anatomical hypertrophy, as evidenced by heart weight-to-body weight ratio, indicative of pressure overload. In contrast, ANP expression within the LV was augmented markedly, but only at the higher dose of 1-NAME, in agreement with previous findings (5, 57). Zhang et al. (63) also observed increased expression of ANP mRNA, but only in a subgroup of animals in which LVH was evident and expression of renin and ACE mRNAs was increased after administration of high doses of 1-NAME. Expression of ANP may represent a response to, and be dependent on the extent of, activation of the renin-angiotensin system and manifestation of LVH, rather than pressure overload per se.

Expression of AM and receptor components. Expression of AM was increased significantly and in a dose-dependent manner within cardiomyocytes from the LV only, even in the absence of cardiac hypertrophy, after administration of low and moderate doses of 1-NAME, implying a progressive adaptation to increasing severity of pressure overload, rather than to NO deficiency per se. Increased expression of the peptide also has been observed in the LV of stroke-prone SHR (SHR-SP, a model of malignant hypertension; Ref. 61), during LVH and the transition to heart failure in Dahl salt-sensitive rats (34), in congestive heart failure following coronary artery ligation (59), and in ischemic cardiomyopathy induced by infusions of isoproterenol (40). Infusion of AM reduced the LV mass-to-body mass ratio after pressure-overload hypertrophy induced by aortic banding in rats, whereas infusion of hydralazine, given at a dose designed to lower blood pressure to a similar extent, did not (28). In addition to the established vasorelaxant and natriuretic effects (30, 41), these data imply a possible direct role for AM, produced locally within the heart, in the attenuation of cardiac growth. Cardiomyocytes express the precursor peptide mRNA (this study) and secrete the mature AM peptide into culture medium (17, 29). This expression is enhanced in response to hypertrophic growth stimuli including mechanical stretch (60), hypoxic stress (32), cytokines (60), and angiotensin II (32). AM attenuates protein synthesis (Ref. 50; 60; present study) and phenotypic changes in gene expression (50) in response to hypertrophic stimuli. Enhanced expression of AM may provide a local compensatory response to increased mechanical stretch, enhanced oxidative stress, and release of hypertrophic mediators, helping to offset cardiomyocyte hypertrophy after administration of 1-NAME.

RAMP2 and RAMP3 mRNA expression was increased significantly, whereas CRLR mRNA tended to increase and RAMP1 mRNA was unchanged in LV cardiomyocytes in the absence of LVH. Enhanced expression of CRLR and RAMP2 mRNA also has been reported in the LV of SHR-SP (61), hypertrophied myocardium of Dahl salt-sensitive rats (together with RAMP3; Ref. 34), ischemic cardiomyopathy (59), and heart failure induced by coronary artery ligation (40). In contrast, Cueille et al. (11) reported enhanced expression of RAMP1 and RAMP3, but not of RAMP2 or CRLR, in aortic banded rats with congestive heart failure. CGRP, released from sensory nerve fibers in response to myocardial ischemia, exerts a direct protective effect against myocardial ischemia-reperfusion injury (42) and initiates hypertrophy of ventricular cardiomyocytes via CGRP1 receptors (8). In contrast, AM attenuates cardiomyocyte hypertrophy (Refs. 50, 60; present study). Upregulation of RAMP2/3 expression would promote the interaction of AM with AM1 and AM2 receptors, rather than CGRP1 receptors, thereby favoring the antihypertrophic effects of AM. It remains to be established whether such effects are mediated by AM1 or AM2 receptors, or both, although the marked upregulation of RAMP3 would support a more prominent role for the AM2 receptor in counterregulation in response to the pathophysiological changes occurring in the myocardium in NO-deficient hypertension. Angiotensin II enhanced expression of both RAMP1 and RAMP3 (25), whereas ET-1 increased expression of CRLR and RAMP3 mRNAs and reduced RAMP2 without affecting expression of RAMP1 in neonatal cardiomyocytes (26). It is possible that activation of hypertrophic growth factors, including angiotensin and ET-1, could account for modulation of AM receptor components in the 1-NAME model. Recently, intermedin, a novel member of the CGRP/AM family, was identified (44) and found to interact nonselectively with RAMPs 1–3, although the physiological significance of this peptide is poorly understood at present. Although intermedin is expressed less abundantly in normal myocardium than AM (unpublished observation), the possibility that this peptide or its interactions with receptor components also may be influenced by NO-deficient hypertension should also be taken into consideration.

In conclusion, 1-NAME enhances protein synthesis in both LV and RV cardiomyocytes but elicits a hypertrophic phenotypic change, manifest as upregulation of the contractile gene, skeletal α-actin, and cardioendocrine peptides ANP, BNP, and ET-1, accompanied by altered expression of the counterregulatory peptide adrenomedullin and its receptor components RAMP2 and RAMP3 in LV only, indicating that the former is due to impaired NO synthesis, whereas the phenotypic changes represent a response to pressure overload.

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GRANTS

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