Predominant activation of endothelin-dependent cardiac hypertrophy by norepinephrine in rat left ventricle

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Received 16 November 2001; accepted in final form 16 January 2002

Moser, Lutz, Jörg Faulhaber, Rudolf J. Wiesner, and Heimo Ehmke. Predominant activation of endothelin-dependent cardiac hypertrophy by norepinephrine in rat left ventricle. Am J Physiol Regulatory Integrative Comp Physiol 282: R1389–R1394, 2002. First published January 24, 2002; 10.1152/ajpregu.00685.2001.—Locally released endothelin (ET)-1 has been recently identified as an important mediator of cardiac hypertrophy. It is still unclear, however, which primary stimulus specifically activates ET-dependent signaling pathways. We therefore examined in adult rats (n = 51) the effects of a selective ETA receptor antagonist in experimental models of cardiac hypertrophy, in which myocardial growth is predominantly initiated by a single primary stimulus. Rats were exposed to mechanical overload (ascending aortic stenosis), increased levels of circulating ANG II (ANG II infusion combined with hydralazine), or adrenergic stimulation (infusion of norepinephrine in a subpressor dose) for 7 days. All experimental treatments significantly increased left ventricular weight/body weight ratios compared with untreated rats, whereas systolic left ventricular peak pressure was increased only after ascending aortic stenosis. ETA receptor blockade exclusively reduced norepinephrine-induced cardiac hypertrophy and atrial natriuretic peptide gene expression. Blood pressure levels and heart rates remained unaffected during ETA receptor blockade in all experimental groups. These data indicate that in rat left ventricle, the ET-dependent signaling pathway leading to early development of cardiac hypertrophy and fetal gene expression is primarily activated by norepinephrine.

endothelin II; endothelin-A receptor; gene expression; norepinephrine; remodeling

ENDOTHELIN (ET) is a potent vasoconstrictor that was first described by Yanagisawa and co-workers in 1988 (35). During the past years, it has become clear that in addition to its hemodynamic effects, ET-1 can also act as a growth-promoting hormone in the myocardium. Initial observations made on isolated cardiomyocytes revealed an increase of protein biosynthesis and cellular volume after the addition of ET-1 to the culture medium (11). Consecutive studies in intact animals demonstrated a stimulation of the cardiac ET system in several forms of extrinsic cardiac hypertrophy (2, 10, 12, 15, 20, 21), particularly during its early development (12, 15, 21). Correspondingly, either selective ETA subtype receptor blockade or unselective ET/AB subtype receptor blockade significantly attenuated early myocardial hypertrophy and fetal gene expression associated with renal artery stenosis (7, 9), suprarenal abdominal aortic banding (12), ANG II infusion (8), and high-dose infusion of norepinephrine (NE) (15). In all of these pathophysiologically relevant models, however, several primary stimuli, which can individually induce myocardial hypertrophy, are activated concurrently, i.e., mechanical load, the renin-angiotensin system, and/or the sympathetic nervous system. Accordingly, it remains unclear whether the cardiac ET system is specifically activated either by one or by a combined action of several primary stimuli or whether it constitutes a more general downstream signaling pathway of myocardial growth.

In the present study, we therefore sought to design experimental paradigms of extrinsically induced cardiac hypertrophy, in which myocardial growth is predominantly initiated by a single primary stimulus. To increase mechanical load to the ventricle, in one group of animals the ascending aorta was banded directly at the root of the aortic arch. In a previous study, we found that this procedure does not stimulate the circulating renin-angiotensin system (33). The observation by others that myocardial growth after ascending aortic stenosis is neither diminished by angiotensin AT1 receptor blockade (32) nor by angiotensin-converting enzyme inhibition (37) strongly suggests that the local cardiac renin-angiotensin system does not participate in the growth response to mechanical overload. To investigate the isolated role of circulating ANG II in ET-dependent cardiac hypertrophy, in a second group of rats ANG II was chronically infused subcutaneously via osmotic minipumps. These animals also received the vasodilator hydralazine to prevent increases in blood pressure induced by the elevation of circulating ANG II levels. This experimental protocol has previously been shown to induce blood pressure-independent myocardial growth (17). There is at present no
evidence indicating any significant contribution of an increased sympathetic activity in these two experimental forms of cardiac hypertrophy. Finally, to initiate cardiac hypertrophy primarily via a stimulation of myocardial α1/β1-adrenoceptors (36), a third group of animals received chronic infusions of NE at a low dose (100 μg·kg body wt·h−1). To assess the participation of the ET system in each of these experimental forms of extrinsic cardiac hypertrophy, animals were treated with either saline or the ETA receptor antagonist LU-135252 (27).

METHODS

Experimental groups. Experiments were conducted on a total of 51 female Sprague-Dawley rats weighing 180–200 g (9 wk old). All surgical procedures were performed under anesthesia with ketamine/xylazine (4 and 100 mg/kg body wt, respectively). Constriction of the ascending aorta was performed as described previously. ANG II was chronically administered at a rate of 200 ng·kg body wt·h−1 via osmotic minipumps (Alzet model 2001, Charles River) that were implanted subcutaneously. In female rats, this dose of ANG II induces a moderate increase in mean arterial blood pressure and significant left ventricular hypertrophy by ~10–15% after 7 days of chronic infusion (34; compare Refs. 17). To prevent changes in blood pressure, hydralazine was given with the drinking water to ANG II-infused rats. The daily intake was 10 mg/kg body wt as calculated from the average consumption of water. NE was chronically administered via osmotic minipumps at a rate of 100 μg·kg body wt·h−1. Preliminary experiments in our laboratory showed that this dose induces left ventricular hypertrophy without affecting systolic or mean arterial blood pressure. All animals survived the experimental interventions and were included in the final analysis.

Chronic ETA receptor blockade was induced by administration of LU-135252 [2-(4,6-dimethoxy-pyrimidin-2-yl)oxy]-3-methoxy-3,3-diphenyl-propionic acid; Knoll AG, Ludwigshafen, Germany), an orally active ETA receptor antagonist. The drug was given by gavage twice daily (25 mg/kg body wt each time, i.e., a total daily dose of 50 mg/kg). Sham-treated rats received saline instead of the antagonist. LU-135252 is a nonpeptide, selective, and long-acting ETA receptor antagonist with a plasma half-life of ~12 h. The selectivity for ETA receptors, expressed as the ratio of the affinities for ETA over ETB receptors, is 131 (Kf for ETA receptors: 1.4 nmol/l; Kf for ETB receptors: 184 nmol/l) (25). A number of recent studies showed that a daily dose of 20–60 mg/kg body wt of LU-135252 given orally to block ETA receptors is effective in blocking a large variety of pathophysiological processes in cardiovascular disease models with an activated ET system (1–4, 6, 7, 22, 28). In normal rats, chronic administration of LU-135252 at a dose of 20 mg/kg body wt·day−1 with the diet resulted in morning plasma levels of LU-135252 of ~30 μmol/l (2). In dogs, oral administration of LU-135252 at doses of 10 and 30 mg/kg body wt completely blocked the pressor effect of intravenous bolus injections of 0.75 nmol/kg ET-1 (24). Accordingly, the administration of 25 mg/kg body wt of LU-135252 twice daily (i.e., every 12 h) by gavage should provide a high degree (>99%) of ETA receptor blockade.

At the end of the experimental period of 7 days, animals were anesthetized and left ventricular peak pressure or systolic aortic blood pressure was measured by directly puncturing the chamber from the abdominal cavity through the diaphragm or via a catheter inserted into the left A. femoralis and advanced into the abdominal aorta. Heart rates were derived from the direct pressure signal. After completion of the hemodynamic measurements, animals were killed and ratios of left ventricular weight to body weight were determined as an estimate of left ventricular hypertrophy. Then left ventricular tissue was frozen with liquid nitrogen and stored at −80°C for determination of levels of atrial natriuretic peptide (ANP) mRNA. All experiments were conducted in accordance with institutional guidelines and the Guide for the Care and Use of Laboratory Animals put forth by the U.S. Department of Health and Human Services, National Institutes of Health Publication No. 86–23, and were approved by local authorities.

RNA analysis. RNA was extracted from ventricles pulverized under liquid nitrogen (5). It was confirmed that the probes used for mRNA analysis hybridized to a single band of the appropriate molecular weight by Northern blot analysis (33). For quantification, RNA was blotted to nitrocellulose in serial dilutions (4, 2, and 1 μg RNA/slot) using a vacuum filtration slot blot apparatus. Blots were probed consecutively with cDNA probes specific for ANP mRNA (plasmid containing a 145-bp, PCR-derived sequence of rat preproANP mRNA was kindly donated by Prof. Forssmann, Hannover, Germany) and 28S rRNA under conditions described in detail previously (7). Autoradiographs of the slot blots were scanned densitometrically, and tissue levels of ANP mRNA were expressed as arbitrary densitometric units/28S densitometric units taking care that the signal was in the linear range for all measurements.

Statistical analysis. Statistical analysis was performed using Graph-pad prism software. For a statistical evaluation of the effects of the different treatments compared with sham-treated animals, blood pressure data and ratios of left ventricular weight to body weight were analyzed by one-way ANOVA followed by the Bonferroni test. Statistical analysis of the effects of ETA receptor antagonism was made separately for each group by the two-tailed, unpaired Student’s t-test. All data are expressed as means ± SE. A value of P < 0.05 was considered significant.

RESULTS

The effects of the different experimental interventions to induce cardiac hypertrophy are summarized in Table 1. Banding of the ascending aorta significantly increased left ventricular peak pressure from 115 ± 5 to 182 ± 7 mmHg (P < 0.001; n = 8). On the contrary, blood pressure was neither elevated by chronic infusion of ANG II combined with hydralazine nor by

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<tr>
<th>Parameter</th>
<th>Sham</th>
<th>AAS</th>
<th>ANG II</th>
<th>NE</th>
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<tr>
<td>Blood pressure, mmHg</td>
<td>115 ± 5</td>
<td>182 ± 7 †</td>
<td>118 ± 7</td>
<td>116 ± 8</td>
</tr>
<tr>
<td>LV weight/body weight, mg/g</td>
<td>2.06 ± 0.03</td>
<td>2.87 ± 0.05 †</td>
<td>2.44 ± 0.07 †</td>
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<tr>
<td>ANP/28S</td>
<td>1.42 ± 0.10</td>
<td>7.73 ± 2.01 ‡</td>
<td>2.09 ± 0.14</td>
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Values are means ± SE; n, number of animals. Blood pressures refer to systolic peak pressures determined either in the left ventricle [aorta ascendens stenosis (AAS)] or abdominal aorta [ANG II + hydralazine, norepinephrine (NE)]. ANP, atrial natriuretic peptide.

*P < 0.05, †P < 0.001 by 1-way ANOVA followed by Bonferroni’s multiple comparison test. LV, left ventricular.
chronic low-dose infusion of NE. All three experimental treatments caused significant left ventricular hypertrophy, as indicated by increased ratios of left ventricular weight to body weight. None of the interventions caused a decrease in body weight (data not shown). Left ventricular hypertrophy was most pronounced in the ascending aortic banding group (39 vs. 18% in the ANG II-infused group and 24% in the NE-infused group). ANP gene expression was elevated in all groups. Interestingly, ANP-to-28S ratios were considerably higher after aortic banding and chronic NE infusion than after ANG II combined with hydralazine (where the difference failed to reach statistical significance), indicating that different primary hypertrophic stimuli induce different cardiac gene expression patterns.

The effects of ET$_A$ receptor blockade on the increase in left ventricular mass and ANP gene expression induced by the different experimental interventions are depicted in Figs. 1–3. ET$_A$ receptor blockade had no effects on body weight in any of the experimental groups (data not shown). In ascending aortic-banded animals, ET$_A$ receptor blockade had no effects on the development of left ventricular hypertrophy or myocardial expression of the ANP gene compared with animals treated with saline (Fig. 1). Similarly, left ventricular hypertrophy and ANP gene expression remained unaffected by ET$_A$ receptor blockade in animals subjected to ANG II combined with hydralazine (Fig. 2). In contrast, both increase in left ventricular mass as well as stimulation of left ventricular ANP gene expression were significantly reduced by >50% in rats with NE-induced cardiac hypertrophy (Fig. 3). In all three treatment groups, chronic ET$_A$ receptor blockade by LU-135252 remained without effects on blood pressure and heart rate (Figs. 1–3).

![Fig. 1. Effects of endothelin (ET)A receptor blockade by LU-135252 compared with saline infusion on left ventricular (LV) peak pressure (A), heart rate (B), the ratio of LV weight to body weight (C), and the ratio of atrial natriuretic peptide (ANP) mRNA to 28S rRNA (ANP/28S; D) in rats subjected to ascending aortic stenosis for 7 days. Data from each group are presented as means ± SE.](http://ajpregu.physiology.org/)

![Fig. 2. Effects of ET A receptor blockade by LU-135252 compared with saline infusion on systolic blood pressure (A), heart rate (B), the ratio of LV weight to body weight (C), and the ratio of ANP/28S (D) in rats subjected to infusion of ANG II combined with hydralazine (10 mg·kg body wt$^{-1}$·day$^{-1}$) for 7 days. Data from each group are presented as means ± SE.](http://ajpregu.physiology.org/)
significantly attenuated NE-induced cardiac growth.

In contrast, blockade of ET A receptors by infusion of norepinephrine in a subpressor dose (100 μg/kg body wt) was effective in blocking vascular remodeling (6, 8, 22) and ANP gene expression induced by ANG II. Although the increase in protein synthesis induced by ANG II was partially blocked by either selective ETA receptor blockade, anti-sense oligonucleotides against preproET-1, or AT1 receptor blockade (11). These results suggest that locally released ET-1 may act as an important mediator of ANG II-induced myocardial growth. Further in vivo studies demonstrated that ET antagonism was very effective in blocking vascular remodeling (6, 8, 22) and that AT1 receptor antagonism significantly reduced this hypertensive effect of ETA receptor blockade in rats with renovascular hypertension (6, 8, 22, 26). Similarly, cardiac hypertrophy associated with renovascular hypertension was largely blunted by administration of a selective ETA receptor antagonist, particularly during the early pressure-independent phase (7). Long-term ETA receptor blockade in rats with renovascular hypertension did not affect blood pressure or cardiac hypertrophy, but it completely prevented vascular remodeling of intramyocardial arteries (9). Again, it is important to note that renovascular hypertension does not represent a condition with a pure stimulation of the renin-angiotensin system, but it is associated with a marked increase of sympathetic activity and the release of NE in both experimental animal models (16) as well as humans (13). The present observations of a lack of effect of ETA receptor blockade on left ventricular hypertrophy and ANP gene expression induced by a “pure” elevation of circulating ANG II levels may therefore suggest that the inhibitory influences of ET

DISCUSSION

The present results demonstrate that neither an increase in mechanical load nor elevated levels of ANG II without concurrent changes in blood pressure induces cardiac hypertrophy or ANP gene expression via the ETA receptor-signaling pathway within 7 days after induction. In contrast, blockade of ETA receptors significantly attenuated NE-induced cardiac growth and ANP gene expression. These data suggest that the myocardial ET signaling pathway mediating early cardiac hypertrophy and ANP gene expression is primarily activated by NE.

The finding that cardiac hypertrophy caused by an increase in mechanical load was unaffected by ETA receptor blockade seems to be at variance with an earlier study by Ito et al. (12) who observed a significant reduction of cardiac remodeling by the selective ETA receptor blocker BQ-123. In contrast to the present study, these investigators used suprarenal abdominal aortic banding, which resembles the experimental model of one-kidney, one-clip hypertension, to induce an elevation of blood pressure. In dogs, suprarenal abdominal aortic banding has been shown to activate the renin-angiotensin system, whereas plasma renin activity (PRA) did not change after ascending aortic banding because of an elevated cardiac filling pressure causing an increased release of ANP and a stimulation of cardiac mechanoreceptors (18). In a previous study, we could also show that in rats subjected to ascending aortic stenosis, PRA remains unchanged (33). Because PRA was not determined by Ito et al. (12), it remains unclear whether suprarenal abdominal aortic banding directly increased left ventricular peak pressure similar to ascending aortic banding or whether the hypertension was secondary to an activation of the renin-angiotensin system and/or fluid retention. The rather slow increase in blood pressure (≥24 h) after abdominal banding reported by Ito et al. (12), however, speaks in favor of the latter alternative.

Most unexpectedly, ETA receptor blockade also had no effects on cardiac hypertrophy and ANP gene expression induced by ANG II. Several recent studies strongly implicated a close interplay between ANG II and ET in the regulation of cardiac growth. In cultured neonatal cardiomyocytes, ANG II upregulated ET gene expression and ET-1 release at a dose that induced cellular hypertrophic growth (11). The increase in protein synthesis induced by ANG II was completely blocked by either selective ETA receptor blockade, antisense oligonucleotides against preproET-1, or AT1 receptor blockade (11). These results suggested that locally released ET-1 may act as an important mediator of ANG II-induced myocardial growth. Further in vivo studies demonstrated that ET antagonism was very effective in blocking vascular remodeling (6, 8, 22) and enhanced vascular responsiveness (6, 26) in chronically ANG II-infused rats. In these studies, however, blood pressure increased considerably in response to ANG II, and ET antagonism significantly reduced this hypertension (6, 8, 22, 26). Similarly, cardiac hypertrophy associated with renovascular hypertension was largely blunted by administration of a selective ETA receptor antagonist, particularly during the early pressure-independent phase (7). Long-term ETA receptor blockade in rats with renovascular hypertension did not affect blood pressure or cardiac hypertrophy, but it completely prevented vascular remodeling of intramyocardial arteries (9). Again, it is important to note that renovascular hypertension does not represent a condition with a pure stimulation of the renin-angiotensin system, but it is associated with a marked increase of sympathetic activity and the release of NE in both experimental animal models (16) as well as humans (13). The present observations of a lack of effect of ETA receptor blockade on left ventricular hypertrophy and ANP gene expression induced by a “pure” elevation of circulating ANG II levels may therefore suggest that the inhibitory influences of ET

**Fig. 3.** Effects of ETA receptor blockade by LU-135252 (50 mg·kg body wt⁻¹·day⁻¹; n = 6) compared with saline infusion (n = 6) on systolic blood pressure (A), heart rate (B), the ratio of LV weight to body weight (C), and the ratio of ANP/28S (D) in rats subjected to infusion of norepinephrine in a subpressor dose (100 μg·kg body wt⁻¹·h⁻¹) for 7 days. Data from each group are presented as means ± SE.
antagonism found in these former studies may have been secondary to alterations in blood pressure and/or sympathetic activity.

A reduction of cardiac hypertrophy induced by chronic NE infusions was also recently found after unselective ETA/B receptor blockade by bosentan (15). In contrast to the present study, however, left ventricular ANP gene expression remained unaffected. One possible explanation for this discrepancy may be related to differences in ET antagonism (selective vs. nonselective blockade). Alternatively, the much higher dose of 600 µg·kg body wt⁻¹·h⁻¹ of NE used by Kadoura and co-workers (15) may have elicited different responses in myocardial gene expression. In preliminary experiments, we found that chronic infusion of NE at this rate was lethal within 48 h after surgery in 80% of infused rats. In the surviving animals, we observed a very pronounced increase in left ventricular weight-to-body weight ratios and marked increases in blood pressure by 40–50 mmHg. Unfortunately, blood pressures were not reported by Kadoura and co-workers (15). Also, in the present investigation, blood pressures were only determined at a single time point during anesthesia. Because cardiac unloading can cause a rapid reduction of left ventricular mass even in the presence of intense neurohumoral stimulation by ET-1 (19), further experiments in awake rats need to be performed to clarify this issue.

A limitation of the present study is that it does not differentiate between effects on cardiomyocytes, fibroblasts, and extracellular matrix. Accumulating evidence suggests that ET-dependent signaling may significantly affect cardiac fibrosis in various forms of cardiac hypertrophy (2, 9, 23). It seems very likely that the primary stimuli investigated here (mechanical load, ANG II, and NE) contribute to different extents to those different aspects of cardiac remodeling. Their individual precise effects on collagen deposition, increase in extracellular matrix, and activation of cardiac fibroblasts cannot be estimated from the present study. It should be noted, however, that the increase in interstitial volume observed in early stages of cardiac hypertrophy does not exceed ~1–2% of total volume (2, 9, 32). In the present study, left ventricular weight-to-body weight ratios increased by 18–39% (Table 1). This indicates that most of the increase in left ventricular mass in response to all three primary stimuli can be attributed to cardiomyocyte hypertrophy.

**Perspectives**

The present findings imply a major role for an interaction between the sympathetic nervous system (or circulating catecholamines) and the myocardial ET system during the early development of cardiac hypertrophy. This is particularly interesting from a pathogenic point of view, as several studies suggest that ET-1 may act as a “triggering factor” for myocardial growth (12, 15, 21). Isolated cardiac myocytes display a hypertrophic response with α-adrenergic stimulation (30, 31), and it has recently been shown that the α₁-adrenoceptor agonist phenylephrine induces ET-1 gene expression and accelerates the conversion of big ET to bioactive ET-1 in cultured neonatal cardiomyocytes (14). Stimulation of the cardiac ET system with adrenergic activation therefore appears to constitute an important signaling pathway of early myocardial growth, which may initiate cardiomyocyte hypertrophy even in pathophysiological states without obvious catecholamine excess such as renal artery stenosis (7). The therapeutic value of ET antagonism for the treatment of cardiac hypertrophy, however, remains to be clarified. Although blocking the ET system has been shown to prevent excessive myocardial hypertrophy in cardiac failure (29), it may also be harmful under pathophysiological conditions such as myocardial infarction, when early compensatory cardiac growth induced by adrenergic stimulation is required.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 320, C5 to R. Wiesner) and Knoll AG, Ludwigshafen, Germany (to H. Ehmke). J. Faulhaber received a scholarship from the Graduiertenkolleg “Experimentelle Nieren- und Kreislauforschung.”

**REFERENCES**


