Carvedilol and Lacidipine Prevent Cardiac Hypertrophy and Endothelin-1 Gene Overexpression After Aortic Banding

Pierre-Emmanuel Massart, Julian Donckier, Jan Kyselovic, Théophile Godfraind, Guy R. Heyndrickx, Maurice Wibo

Abstract—Carvedilol and lacidipine have been shown to exert cardioprotective effects in rat models of chronic hypertension. We investigated their effects in an acute model of pressure overload produced by suprarenal aortic constriction, in which enhanced myocardial production of endothelin-1 could play a crucial role. In the absence of drug treatment, after 1 week, aortic banding provoked an increase in carotid pressure associated with left ventricular hypertrophy (29%; \(P<0.01\)). These changes were accompanied by increased myocardial expression of preproendothelin-1 (2.5 times; \(P<0.05\)) and skeletal \(\alpha\)-actin (3.6 times; \(P<0.05\)), but the expression of cardiac \(\alpha\)-actin was not modified. Oral administration of carvedilol at a dose of 30 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) to rats with aortic banding normalized carotid pressure and left ventricular weight as well as preproendothelin-1 and skeletal \(\alpha\)-actin gene expression. Carvedilol at a lower dose (7.5 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\)) and lacidipine 1 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) had only moderate and nonsignificant effects on carotid pressure but largely prevented left ventricular hypertrophy (\(P<0.01\)) and preproendothelin-1 overexpression (\(P<0.05\)). Labetalol (60 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\)) tended to exert similar effects but insignificantly. These results show that the antihypertensive properties of carvedilol and lacidipine are partly independent of their antihypertensive effects and may be related to their ability to blunt myocardial preproendothelin-1 overexpression. Moreover, carvedilol at a dose of 7.5 mg \(\cdot\) kg\(^{-1}\) \(\cdot\) d\(^{-1}\) did not prevent myocardial overexpression of skeletal \(\alpha\)-actin, which suggests that, in this model, reexpression of a fetal gene can be activated by pressure overload independently of cardiac hypertrophy.

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Key Words: endothelin ■ hypertrophy, left ventricular ■ hypertension, experimental ■ \(\alpha\)-actin ■ carvedilol ■ lacidipine

Endothelin-1 (ET-1), a potent vasoconstrictor peptide produced by endothelial cells,\(^1\) is endowed with remarkable growth-promoting properties. In cultured neonatal cardiac myocytes, ET-1 induces hypertrophy,\(^2\)\(^-\)\(^3\) whereas other stimuli, such as mechanical stretch or angiotensin II (Ang II), may evoke hypertrophic growth by increasing ET-1 biosynthesis.\(^4\)\(^5\) ET-1 has also been shown to play an important role in some in vivo models of cardiac hypertrophy. ET-1 receptor antagonists prevent, at least transiently, left ventricular (LV) hypertrophy evoked by suprarenal aortic banding.\(^6\) ET-1 receptor blockade has been reported to attenuate cardiac hypertrophy in stroke-prone spontaneously hypertensive rats (SHRSP) fed a high-salt diet,\(^7\)\(^8\) in uninephrectomized rats receiving deoxycorticosterone acetate and a high-salt diet,\(^9\)\(^10\) in renovascular hypertensive rats,\(^11\)\(^12\) and in rats infused with Ang II\(^12\) or norepinephrine.\(^13\)

Carvedilol is a nonselective, vasodilating \(\beta\)-blocker with potent antioxidant and free radical–scavenging properties\(^14\) that is used in the treatment of hypertension, angina, and congestive heart failure.\(^15\) Unlike other \(\beta\)-blockers, carvedilol reduces ET-1 biosynthesis in cultured endothelial cells.\(^16\)\(^17\) Recently, carvedilol, at doses that do not reduce systemic blood pressure, has been reported to prevent cardiac growth and remodeling in SHRSP maintained on 1% NaCl drinking solution and high-fat diet.\(^18\) In SHRSP, the calcium channel blocker lacidipine also prevents salt-dependent cardiac hypertrophy at doses that only minimally affect hypertension, and this effect could be related to a concomitant reduction in myocardial levels of preproendothelin-1 (preproET-1) mRNA.\(^19\)\(^20\) The present study was therefore conducted to investigate the effects of carvedilol and lacidipine on LV growth and preproET-1 gene expression in rats subjected to hemodynamic overload produced by suprarenal aortic constriction, a model of LV hypertrophy in which myocardial ET-1 appears to play a key role, at least in the acute stage.\(^6\)\(^21\) Rats subjected to aortic banding and treated with labetalol were included in this study, because this drug shares with carvedilol the property to inhibit both \(\alpha\)- and \(\beta\)-adrenoceptors.

Methods

Experimental Animals

All procedures followed were in accordance with institutional guidelines. Sixty Sprague-Dawley male rats (OFA, Iffa Credo, L’arbresle,
France) weighing 240 to 260 g were divided randomly into 6 groups: control (sham-operated) rats (sham); rats with aortic banding that received ordinary food (AOB); and 4 groups of rats with aortic banding that received ordinary food mixed with one of the following: a high dose of trimethobenzamide (Carvedilol (CAR) 30 mg \cdot kg^{-1} \cdot d^{-1}), a low dose of carvedilol (CAR 1 mg \cdot kg^{-1} \cdot d^{-1}), lacidipine (LACI 1 mg \cdot kg^{-1} \cdot d^{-1}) or labetalol (LABE 60 mg \cdot kg^{-1} \cdot d^{-1}). Aortic constriction was performed as initially described by Jouannot and Hatt, and with minor modifications. Briefly, animals were anesthetized with 2,2,2-trimethobenzamide (Sigma Chemical Co) at a dose of 0.025 g/100 g body weight. Under sterile conditions, the abdominal aorta was exposed through a midline abdominal incision and constricted at the suprarenal level using a 25-gauge needle (outside diameter, 0.5 mm) to establish the diameter of ligature. A similar procedure was performed for sham except for the ligature. Drug treatment was started 1 day before surgery, and the daily intake of drug-containing food was monitored. One week after surgery, animals were anesthetized with 2,2,2-trimethobenzamide, the right carotid and femoral arteries were cannulated with a polyethylene catheter connected to a Statham transducer (P23, Gould Inc) and the mean carotid and femoral arterial pressures were measured. Thereafter, blood samples were collected and animals were killed by exsanguination. Hearts were rinsed with cold Krebs solution, and left ventricles were collected separately, weighed, and immediately frozen in liquid nitrogen.

### Plasma Immunoreactive ET-1 and Plasma Renin Activity

Plasma ET-1 was extracted using Sep-Pack C18 cartridges (Waters Associates) and measured by radiomunoassay, as previously reported. Plasma renin activity (PRA) was measured according to a standard procedure (Medix Biochemica). Because anesthesia and arterial pressure measurement evoked elevation of PRA (reference 24 and data not shown), care was taken to collect blood samples under strictly comparable conditions.

### Isolation of mRNA and Northern Blot Analysis

mRNA (polyA+ RNA) was isolated from frozen ventricles ground in liquid nitrogen with the use of a commercially available kit (Fast Track 2.0 kit, Invitrogen). Samples of 5 to 10 μg of polyA+ RNA were analyzed by Northern blotting with either cDNA probes (preproET-1 and GAPDH) or synthetic oligonucleotide probes (skeletal and cardiac ß-actin) labeled with 32P, as described previously. Radioactive spots were detected and quantified by means of the Cyclone storage phosphor system (Packard Instrument), and they were also visualized on autoradiographic film. Amounts of mRNA species were expressed relative to GADPH mRNA, which was taken as internal reference, to correct for differences in RNA loading or transfer. In each Northern blotting experiment, results were normalized with respect to sham.

### Statistical Methods

Results are expressed as mean±SEM. Differences between groups were assessed using nonparametric Kruskal-Wallis and Mann-Whitney tests. Rank correlation coefficients (r) were determined and evaluated by the Spearman test. Probability values <0.05 were considered significant.

### Results

#### Biometric and Hemodynamic Data and Plasma Levels of Renin and ET-1

In the absence of drug treatment, mean carotid pressure and carotid-femoral pressure gradient were markedly increased at 7 days after aortic banding (Table; P<0.005, AOB versus sham). Heart rate was somewhat lower in AOB than in sham (P<0.05). The average LV/body weight ratio was increased by 29% in AOB (P<0.005), whereas the right ventricular weight was not significantly modified (not shown). Aortic banding was also associated with higher PRA and plasma ET-1 levels at 7 days (P<0.05).

In rats with aortic banding that received carvedilol 30 mg \cdot kg^{-1} \cdot d^{-1} (CAR 30 mg \cdot kg^{-1} \cdot d^{-1}) and labetalol (60 mg \cdot kg^{-1} \cdot d^{-1}) were almost as effective as carvedilol 30 mg \cdot kg^{-1} \cdot d^{-1} for prevention of the increase in LV/body wt ratio induced by aortic banding (P<0.01). However, low-dose carvedilol and labetalol showed only moderate effects on hemodynamic changes, which did not reach significance. These drug treatments reduced PRA values (P<0.05) and also slightly reduced plasma ET-1 levels.

The LV/body wt ratio of LABE was intermediate between those of sham and AOB, and the mean value of LABE was normalized (P<0.005 versus AOB) and heart rate was significantly lowered (P<0.05). The increase in LV/body wt ratio was almost completely prevented by the high dosage of carvedilol (P<0.005) and the rise in plasma ET-1 and PRA was abolished (P<0.05 and P<0.01, respectively). Carvedilol at a lower dose (7.5 mg \cdot kg^{-1} \cdot d^{-1}) and lacidipine (1 mg \cdot kg^{-1} \cdot d^{-1}) were used as an antihypertrophic effect of low-dose carvedilol. Despite this, the hemodynamic changes evoked by labetalol as well as its effects on plasma levels of renin and ET-1 tended to be greater than those evoked by low-dose carvedilol.

### Myocardial Levels of PreproET-1 and ß-Actin mRNA

As illustrated in Figures 1A and 2A, myocardial levels of preproET-1 mRNA were on an average 2.5-fold higher in...
AOB than in sham ($P<0.005$). This overexpression was almost totally abolished by high-dose carvedilol ($P<0.01$; CAR$_{hd}$ versus AOB) and was prevented to a considerable extent by low-dose carvedilol and lacidipine (both $P<0.05$), whereas the effect of labetalol was smaller and did not reach significance. Myocardial levels of skeletal and cardiac $\alpha$-actin mRNA are shown in Figures 1B and 2B. The cardiac isoform was similarly expressed in the various groups. In contrast, the expression of the skeletal isoform was increased 3.6-fold in AOB compared with sham ($P<0.005$). This increase was almost totally prevented by high-dose carvedilol ($P<0.05$) and partially prevented by lacidipine ($P<0.05$), but it was modified by neither low-dose carvedilol nor labetalol.

**Correlation Among LV Weight, Mean Carotid Pressure, and mRNA Levels**

As illustrated in Figure 3, which shows data from the (drug-treated and untreated) animals with aortic banding, a significant correlation was found between preproET-1 mRNA levels and LV/body wt ratio on the one hand (Figure 3A; $r_s=0.43$, $P<0.01$) and between skeletal $\alpha$-actin mRNA levels and mean carotid pressure on the other (Figure 3B; $r_s=0.65$, $P<0.0001$). In contrast, no correlation was detected between preproET-1 mRNA and mean carotid pressure or between skeletal $\alpha$-actin mRNA and LV/body wt ratio.

**Discussion**

In agreement with previous studies,$^{6,21,25}$ we found that pressure overload induced by suprarenal aortic constriction evoked LV hypertrophy, reexpression of a fetal gene, and preproET-1 overexpression. Increased myocardial levels of preproET-1 mRNA as well as increased plasma levels of ET-1 were observed at 7 days after aortic banding, in line with previous reports.$^{21,25}$ Myocardial preproET-1 overexpression was no longer detectable at 2 weeks, whereas LV hypertrophy and increased expression of skeletal $\alpha$-actin did not decrease (data not shown). Ito et al$^6$ reported that plasma levels of ET-1 and preproET-1 mRNA levels in LV were maximally increased at 1 day after aortic banding and returned to normal values at 4 days, whereas LV hypertrophy and enhanced expression of fetal genes persisted at 2 weeks. Thus, LV ET-1 overexpression is an early and transient event in this model of acute pressure overload as well as in ventricular hypertrophy induced by norepinephrine infusion in vivo.$^{11}$ The earlier normalization of LV preproET-1 mRNA levels in the experiments of Ito et al$^6$ might be linked to a lower degree of LV hypertrophy (17% increase in LV/body wt ratio at 7 days versus 29% in our study). ET-1 is considered to play a crucial role at an early stage in acute models of cardiac hypertrophy, essentially because selective inhibitors of ET-1 receptors are able to prevent, at least transiently, LV growth in these models in the absence of any significant effect on aortic pressure.$^{6,11}$ Similarly, we show here that low-dose carvedilol and lacidipine had only moderate and nonsignificant effects on mean carotid pressure but abolished the rise in LV weight (Table) and the increase in LV preproET-1 gene expression (Figure 2). Moreover, taking into account the data that we obtained from all rats with aortic banding, LV weight at 7 days correlated with LV...
preproET-1 mRNA level (Figure 3A) but not with mean carotid pressure. Thus, in this experimental model, low-dose carvedilol and lacidipine could inhibit myocardial growth primarily by blunting myocardial overexpression of preproET-1 rather than by reducing hemodynamic stress. Regarding the action of lacidipine, this drug has been shown previously to prevent salt-dependent LV hypertrophy and preproET-1 overexpression in SHRSP while producing a limited reduction in high blood pressure.19,20

Besides ET-1, Ang II is considered to be an important mediator of cardiac remodeling evoked by mechanical stress.26 As reported by Baker et al,27 administration of enalapril to rats with aortic banding completely prevents the increase in LV mass at 7 and 15 days, although carotid artery pressure is not reduced. Similar protective effects can be obtained with an antagonist of Ang II receptors.28 In vitro studies indicate that Ang II may promote cardiomyocyte hypertrophy partly through ET-14 and point to the possible importance of interactions between different types of cardiac cells in this respect.29–31 Our finding of higher PRA values at level and mean carotid pressure (B; r = 0.43, P < 0.01) and between skeletal α-actin mRNA level and mean carotid pressure (B; r = 0.65, P < 0.0001) in rats with aortic banding (with or without drug treatment).

In conclusion, the present study shows that carvedilol and lacidipine are endowed with cardiac antihypertrophic properties that are partially independent of their antihypertensive effect and appear to be related to their capacity to decrease myocardial preproET-1 overexpression induced by pressure overload.
Cardiac Hypertrophy, Carvedilol, and Lacidipine

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