Survival Benefits of Different Antiadrenergic Interventions in Pressure Overload Left Ventricular Hypertrophy/Failure

Stefano Perlini, Ivana Ferrero, Giuseppina Palladini, Rossana Tozzi, Chiara Gatti, Monia Vezzoli, Francesca Cesana, Maria Bianchi Janetti, Francesca Clari, Giuseppe Busca, Giuseppe Mancia, Alberto U. Ferrari

Abstract—We observed previously that in rats with aortic banding (Bd), development of left ventricular (LV) hypertrophy is opposed by β-blockade, whereas interventions interfering with α-adrenoceptor function also inhibit interstitial fibrosis. To assess whether these differential structural effects do translate into different effects on LV function and on heart failure mortality, Bd or sham Bd 8-week–old rats were randomized to vehicle treatment (Vh), chemical sympathectomy ([Sx] 6-hydroxydopamine, 150 mg/kg IP twice a week), β-adrenoceptor blockade (propranolol [Pro], 40 mg/kg per day PO), or α-adrenoceptor blockade (doxazosin [Dox], 5 mg/kg per day PO). After monitoring survival for 10 weeks, the survivors were anesthetized to undergo echocardiography and intraarterial blood pressure measurement. Bd-Vh rats showed increased LV and lung weights, as well as LV dilatation, depressed endocardial and midwall fractional shortening and a restrictive transmitral diastolic flow velocity pattern. Compared with Bd-Vh rats, all of the actively treated Bd rats showed less LV hypertrophy, LV dilatation, and lung congestion but no less depression of midwall fractional shortening. In contrast, Sx and Dox but not Pro treatment were also associated with lesser degrees of diastolic dysfunction and, even more importantly, with a striking increase in survival (sham banded rats, 100%; Bd-Vh, 40%; Bd-Pro, 51%; Bd-Sx, 83%; and Bd-Dox, 82%). Although Pro, Sx, and Dox provide similar midterm protection from development of LV hypertrophy and dysfunction and from circulatory congestion, only Sx and Dox favorably affected mortality. These findings indicate that in the aortic banding rat model, α-adrenoceptors are importantly involved in the pathogenesis of cardiovascular deterioration and disease progression. (Hypertension. 2006;48:93-97.)

Key Words: heart failure ■ hypertrophy ■ sympathectomy ■ rats ■ receptors, adrenergic alpha ■ ventricular function, left

H ypertensive heart disease is well known to be accompanied by an exaggerated sympathetic drive to the cardiovascular system1–3: although initially thought to represent a compensatory adjustment able to support the functioning of a mechanically overloaded heart, sympathetic overactivity is now perceived as an inappropriate response, which exerts adverse effects and contributes to disease progression and functional deterioration from compensated left ventricular (LV) hypertrophy to LV dysfunction, overt heart failure, and death.4–6 This is supported by evidence that interfering with cardiac adrenergic drive by β-adrenoceptor blockers has favorable influences at all stages of the disease and prolongs survival.7–9

One of the effects of β-blockers is to oppose the development of myocardial hypertrophy, although in recent studies we observed that this antihypertrophic action has the peculiar feature of opposing the growth of the cardiomycocyte but not the development of interstitial fibrosis,10,11 which was, in contrast, markedly inhibited by chemical sympathectomy or by an α-blocker,12 suggesting a more extensive protective effectiveness by treatments that involve interference with α-adrenoceptors. The above findings, however, were only of histological nature. We, therefore, set out to investigate the effects of different antiadrenergic interventions on the course of hypertensive heart disease, with the aim of establishing whether their differential effects on LV structure are paralleled by differential effects on LV function and/or on animals’ survival.

Methods

Animal Preparation and Surgery

All of the procedures involving animal care were conducted in accordance with the institutional guidelines and following the international policies defined in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No 85-23, 1996). The experiments were performed on 8-week–old Sprague–Dawley male outbred rats, weighting 200 g (Charles River) in which anesthesia was induced by intraperitoneal ketamine (75 mg/kg) plus xylazine (15 mg/kg) and LV pressure overload was created by banding the abdom-
inal aorta (Bd) as described previously.\textsuperscript{12,13} A concurrent group of sham-banded animals was also prepared. The animals were housed under controlled environmental conditions, with access to food (Purina Formula Lab Chow 5008) and water ad libitum.

### Experimental Protocol

One day after surgery, animals were randomized to chronic chemical sympathectomy with 6-hydroxydopamine (150 mg/kg IP twice a week [Sx]), \( \beta \)-adrenergic blockade with oral propranolol (40 mg/kg per day [Pro]), \( \alpha \)-adrenergic blockade with oral doxazosin (5 mg/kg per day [Dox]), or vehicle administration (Vh) for 10 weeks, thereby obtaining 8 experimental groups: S-Vh (n = 10), Bd-Vh (n = 25), S-Sx (n = 10), Bd-Sx (n = 18), S-Pro (n = 10), Bd-Pro (n = 21), S-Dox (n = 10), and Bd-Dox (n = 11). After assessing survival for 10 weeks, rats were again anesthetized and subjected to a complete echocardiographic study (see below). Systolic (SBP) and diastolic blood pressure (DBP) was then measured via a polyethylene catheter inserted into the right carotid artery. The catheter was connected to a Statham P23DC pressure transducer (Gould-Statham), the system having a flat frequency response \( \leq 30 \) Hz, and the carotid artery pressure signal was displayed on a chart recorder (Grass 7D polygraph, Grass Instruments). Finally, the animals were killed by an anesthetic overdose, the heart was quickly excised, and the LV and the right ventricle were separated and weighted. The lungs were also quickly excised and weighted. Heart and lung weight were indexed to body weight and expressed as grams per 100-g body weight.

### Echocardiographic Studies

The 2D Doppler echocardiographic studies were performed and analyzed as described previously.\textsuperscript{13} Briefly, the animal was placed in the prone position and scanned via a 12-MHz transducer connected to a Hewlett-Packard Sonos 5500 machine; 2D-guided M-mode echocardiographic images of the LV were obtained in the parasternal short-axis view at the level of the papillary muscles and recorded on strip-chart paper for subsequent analysis. Doppler-derived mitral inflow velocities were obtained in the apical 4-chamber view. Fractional shortening (FS) at the endocardial and midwall levels were calculated as described previously.\textsuperscript{13}

### Statistical Analysis

All of the data are expressed as mean±SE. Cumulative survival curves were generated by the Kaplan–Meier method. Factorial ANOVA followed by the Newman–Keuls post hoc test was used to determine statistical significance between the different experimental groups. A \( P<0.05 \) was used to indicate statistical significance. All of the statistical procedures were performed using the STATVIEW statistical software package.

### Results

#### Structural and Functional Findings in Survivors

**Blood Pressure, Heart Rate, LV Hypertrophy, and Pulmonary Congestion**

As summarized in Table 1, abdominal aortic banding was associated with a significant increase in SBP and DBP, LV weight index, and lung weight index, indicating the development of pressure-overload hypertrophy and of its progression to LV failure. None of the active treatments attenuated the degree of arterial hypertension nor did it significantly affect heart rate. In contrast, the banding-induced LV hypertrophic response was blunted in all of the actively treated compared with the vehicle-treated animals; moreover, all of the active treatments prevented the development of lung congestion, as indicated by lack of any increase in lung weight index.

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<th>LV end-diastolic dimension (mm)</th>
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**Figure 1.** Effects of sympathectomy, \( \beta \)-blockade, or \( \alpha \)-blockade on LV end-diastolic diameter in aortic-banded and sham-banded rats. Data are shown as mean±SEM. \( \square \), vehicle-treated sham-operated (n = 10) and banded (n = 10) animals; \( \square \), sympathectomized sham-operated (n = 10) and banded (n = 15) animals; \( \bullet \), \( \beta \)-blocker treated sham-operated (n = 10) and banded (n = 11) animals; \( \square \), \( \alpha \)-blocker treated sham-operated (n = 10) and banded (n = 9) animals. \( P<0.05 \) vs respective sham-operated control; \( \dagger P<0.05 \) vs vehicle-treated banded animals.
Echocardiographic Data

Aortic banding was associated with LV dilation, as indicated by a significant increase in end-diastolic internal diameter (Figure 1). Chamber as well as myocardial systolic function were depressed, as shown by the decrease in both endocardial and midwall FS (Figures 2 and 3). Diastolic dysfunction was evident as a clear-cut restrictive pattern of the Doppler transmitral flow and by a significant increase in peak early velocities (Figure 4).

All 3 of the active treatments blunted the extent of pressure overload–induced LV dilation. Although none of the interventions was able to prevent banding-induced myocardial systolic dysfunction, as documented by depressed midwall FS, antiadrenergic interventions partially preserved LV diastolic function, as documented by a significantly lower transmitral early velocity compared with vehicle-treated banded animals. However, these favorable effects were only exerted by sympathectomy and α-adrenergic blockade and not by β-blockade.

Survival

Over the 10-week observation period, all of the sham-operated animals survived, whereas a 60% mortality was recorded in vehicle-treated, aortic-banded animals. In all of the dead animals, postmortem examination revealed signs of pulmonary edema, such as multiple punctuated hemorrhages at the lung surface and invasion of the entire lower and upper airways by pink, frothy fluid.

In the aortic-banded animals, sympathectomy substantially reduced mortality, which was down to 17%. A similarly beneficial effect was observed in banded animals chronically treated with doxazosin, whose mortality amounted to only 18% (both P<0.01 versus vehicle-treated, banded animals). In contrast, outcome was only marginally improved in β-blocker–treated, banded animals, whose 49% mortality was not statistically different from the 60% figure observed in vehicle-treated animals. These data are illustrated in Figure 5.

Discussion

The present study demonstrates that in the aortic-banding rat model of hypertensive heart disease, LV hypertrophy, LV dilation, and the severity of heart failure are attenuated by treatments interfering with β-adrenoceptor–mediated influences. The most important finding, however, is that chemical sympathectomy or doxazosin have the additional ability to attenuate diastolic dysfunction and, most importantly, to substantially improve survival. This strengthens the notion that a sizeable proportion of the cardiovascular adverse effects associated with this experimental model of hypertensive heart disease is mediated by sympathetic overactivity. It also provides the novel finding that α– rather than β-adrenoceptors play a predominant role.

Two features of our study deserve to be emphasized. First, the echo assessments, were only performed at the end of the
observation period thus excluding from the comparisons the rats that died prematurely, in which the functional impairment was likely to be the most severe; this suggests that the extent of the functional benefits brought about by the active versus vehicle treatments, as well as by sympathectomy or doxazosin, versus \( \beta \)-blockade might be even larger than we could document. Second, none of the administered drugs was associated with consistent antihypertensive effects, which was, on the one hand, advantageous in relation to the goals of the study, because it suggests that the differential protection exerted by the various drugs was independent of blood pressure changes, but was, on the other hand, somewhat puzzling (although not new, according to previous observations by us\(^{10-12} \) and others\(^ {14,15} \) as to why agents whose antihypertensive properties are well established were in this setting largely ineffective: there is, to our best knowledge, no available mechanistic evidence to account for this phenomenon). It is also to be considered that the blood pressure measurements were performed under anesthesia, which might have concealed some degree of treatment-related blood pressure lowering under conscious conditions: although this can obviously not be excluded, it is at any rate unlikely to have acted as a confounder in the interpretation of our results, because one could hardly conceive that the blood pressure–lowering effect of doxazosin, if any, may have been larger than that of propranolol, yet the functional and survival benefits of the former compared with the latter agent were clear cut and sizeable.

Our findings prompt some further comments on the pathophysiology of hypertensive LV hypertrophy/dysfunction/failure, as well as on the mechanisms underlying the effects of the administered drug therapies. First, the ability of propranolol treatment to blunt the development of LV hypertrophy, chamber dilation, and pulmonary congestion is in line with the notion of the beneficial effect of \( \beta \)-adrenergic blockers in heart failure. It also strengthens the concept, however, that the cardiac sympathetic activation that accompanies hypertensive heart disease is a maladaptive rather than a compensatory adjustment since the earlier stages of the disease.\(^ {11} \) Second, the evidence from our study that sympathectomy as well as \( \alpha \)-receptor blockade are accompanied by cardiac functional benefits and by a striking prolongation of survival indicate that not just \( \beta \)-adrenoceptor- but also \( \alpha \)-adrenoceptor–mediated influences are crucially involved. Third, the observation that in the sympathectomized and doxazosin-treated animals the improved survival was associated with a preserved LV chamber distensibility (paralleled by an antifibrotic effect\(^ {12} \)) suggests that the latter may be causally linked to the former, that is, that diastolic dysfunction may be relevant in the progression and adverse outcome of hypertensive heart disease. This is in a way suggested also by the observation that, in contrast, midwall FS was similar in all of the groups of animals, which means that the improved survival was not paralleled by a preservation of systolic function. We have to emphasize, however, that the hypothesis that diastolic rather than systolic dysfunction is most tightly related to the prognosis of hypertensive heart disease must be taken with caution, because other possibilities cannot be excluded. One may, for example, speculate that the benefit of \( \alpha \)-blockade depends on the attenuation of an excessive sympathetic vasoconstriction in the coronary and/or in the systemic circulation, with favorable consequences on the perfusion of the heart and other vital organs.

Our data may have a clinical implication, that is, that opposing both \( \alpha \)-adrenergic receptor- and \( \beta \)-adrenergic receptor–mediated influences may prevent the onset of the cardiac changes that evolve toward heart failure. It could be argued, however, that it may not be safe to extrapolate animal data to humans and that, in particular, rodents’ hearts have a much greater density of \( \alpha \)-adrenergic receptors than primates’ hearts.\(^ {16} \) Indeed, the use of \( \alpha \)-adrenoceptor blockers in patients with heart failure
has in the past not been accompanied by demonstrable benefits. Nonetheless, the accepted notion that α-blockers fail to provide any significant benefit or may even be harmful in heart failure is largely based on the results of studies using older, short-acting agents that were administered without β-blockers, the use of which was at that time not recommended. Furthermore, the conclusion of a recent trial that in hypertensive patients doxazosin prevented the development of heart failure less effectively than a diuretic has been questioned because of the poorer blood pressure control in the α-blocker group, as well as the lack of proper and certified criteria for the diagnosis of heart failure. New studies with more effective and long-lasting drugs acting against α-receptor influences may be necessary to solve this issue. As to the findings of the present study, they clearly demonstrate that α-receptor–mediated influences predominate over the β-receptor–mediated ones in the abdominal aortic banding rat model of hypertensive heart disease. Further work is needed to extend these observations and to elucidate more in-depth their underlying mechanisms.

Two limitations of our study should be considered. First, our study was designed to explore the preventive efficacy of antihypertensive treatments, that is, their ability to oppose the development of pressure overload–induced alterations. This means that our data may not be safely extrapolated to what would be the benefit of opposing sympathetic hyperactivity after the functional and structural alterations of hypertensive heart disease have already occurred. This question will rather have to be addressed by ad hoc studies in which the onset of treatment will be appropriately delayed. Second, we have so far not examined the effects of the different interventions on humoral factors known to be activated in hypertensive heart disease and to influence its course, such as angiotensin, endothelin, growth factors, cytokines, and so forth; it would, however, not have been feasible, in a study designed to evaluate survival, to subject the animals to repeated blood withdrawals and/or anesthetics: this was also the reason for delaying blood pressure measurements and ultrasound studies until the very end of the observation period.

In conclusion, we demonstrated in the aortic-banded rat that interventions interfering with α-adrenoceptor–mediated influences provide LV functional benefits over and above those provided by β-blockade, especially as far as diastolic alterations are concerned, and, even more importantly, markedly prolong survival, indicating that also the former receptor is crucially involved in the genesis of cardiovascular deterioration and disease progression in this model.

Perspectives

Our demonstration of the involvement of α-adrenoceptor–mediated influences in experimental hypertensive heart disease, as documented by the large LV functional and survival benefits of interventions that interfere with such receptors, prompts the performance of further signaling and molecular studies in experimental models to dissect out the mechanistic aspects of this phenomenon, as well as of investigations extended to the clinical setting, to establish what is the contribution of α-adrenoceptors to disease progression and what may be the benefit of modern α-adrenoceptor blockers in patients with hypertensive heart disease or heart failure at large.

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Disclosures

None.

References

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