JPET Fast Forward. Published on September 27, 2007 as DOI: 10.1124/jpet.107.130237

Effect of long-term heart rate reduction by I_f-current inhibition on pressure-overload-induced heart failure in rats

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Short title: heart rate reduction in heart failure

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Number of text pages: 13

Abstract: 230

Introduction: 316

Discussion: 1500

Number of tables: 4

Number of figures: 3

Number of references: 37

List of abbreviations:

HRR: heart rate reduction; LV: left ventricular; LVEDP: LV end diastolic pressure; ANP: atrial natriuretic peptide; SERCA: sarco-endoplasmic reticulum Ca^{2+}-ATPase

Section assignment: “cardiovascular”.

ABSTRACT

We investigated the effects of long-term heart rate reduction (HRR) on pressure-overload-induced heart failure. Pressure overload of the left ventricle was induced in 21-day-old rats by banding the ascending aorta. HRR was induced for 3 months with ivabradine (n=44), a selective I_f-current inhibitor, 10 mg/kg/d, starting 14 days after banding. Thirty-six control banded rats and 16 sham-operated rats received standard chow. Banding resulted in severe left ventricular (LV) hypertrophy (+55% vs shams, $p<0.001$) and fibrosis, together with a 34% decrease ($p<0.01$) in the LV shortening fraction. Heart rate decreased by 19% in ivabradine-treated rats ($p<0.005$ vs controls). Stroke volume increased (by 17%, $p<0.01$) while cardiac output did not change with HRR. In contrast, HRR resulted in (i) a marked increase in LV filling pressure ($p<0.01$) as well as in atrial, lung, and right ventricular weights (38%, 30%, and 54% respectively $p<0.001$); (ii) a 50% increase in the incidence of pleural/abdominal effusion ($p<0.001$); (iii) 7% and 26% increases in LV hypertrophy and fibrosis, respectively ($p<0.05$); and (iv) a 53% increase in the ANP mRNA level compared with controls ($p<0.001$)., After 3 months of treatment, ivabradine withdrawal normalised the heart rate and reduced LV size and LV filling pressure ($p<0.05$). In conclusion, pure longstanding HRR showed no beneficial effect on LV dysfunction in a rat model of pressure overload-induced LV hypertrophy, and appeared to favour adverse LV remodelling and its congestive consequences.

INTRODUCTION

The heart rate is usually increased in chronic heart failure and correlates positively with mortality (Lechat et al., 2001). The precise role of the heart rate in the pathophysiology of chronic heart failure is poorly understood, but heart rate reduction (HRR) is considered a specific therapeutic target in patients with heart failure (Laperche et al., 1999). Indeed, HRR increases the duration of both left ventricular (LV) filling and diastolic coronary perfusion, which are altered in heart failure. HRR also reduces myocardial O_2 consumption. However, the effects of long-term HRR on chronic heart failure are largely unknown. In patients with heart failure and cardiac pacing, reversal of beta-blockers-induced bradycardia worsened ventricular function (Thackray et al. 2006). Clinical trials of beta-blockers in chronic heart failure do not allow to conclude definitely regarding the intrinsic effect of the associated HRR (Lechat et al., 2001; Gullestad et al., 2005). Indeed, beta-blockers have additional properties, including inhibition of catecholamine-induced myocardial toxicity and decreased renin secretion, and are likely to favorably influence cardiac remodeling.

The class of pure heart-rate-lowering agents -- inhibitors of the cardiac pacemaker I_f current -- provides an opportunity to study the direct effects of HRR in heart failure. Ivabradine is a selective I_f-current inhibitor that induces HRR in a dose-dependent manner, with no direct hemodynamic effects (Simon et al., 1995; Vilaine et al., 2006). In a rat model of myocardial infarction, ivabradine was beneficial by improving LV systolic function, reducing collagen density in the non-infarcted myocardium, and improving myocardial perfusion and the coronary reserve (Mulder et al., 2004; Dedkov et al., 2007).

The effect of pure HRR on pressure-overload-induced heart failure, a clinically relevant situation, has not been investigated. Accordingly, the aim of our study was to examine the effects of ivabradine-induced pure HRR in a rat model of pressure-overload-

induced heart failure in order to assess the impact of long-term pure HRR itself on LV remodeling and function.

METHODS

Animals and treatments

The study was carried out in accordance with the recommendations of the French Accreditation Committee for Laboratory Animal Care (authorization N° 00577). One hundred 3-week-old male Wistar rats (Charles-River, France) underwent banding of the ascending aorta with a titanium clip (0.60 mm internal diameter) as described elsewhere (Feldman et al., 1993). Ninety-two rats survived surgery and were included in the study. The control group comprised 16 sham-operated rats. The animals were given free access to standard powder rodent chow (A04-C10, U.A.R, France) and tap water ad libitum.

Two weeks after surgery the rats were randomized to receive ivabradine (n = 44) or no treatment (n = 36) for the following 3 months. Ivabradine was mixed with the diet to obtain a dose of about 10 mg/kg/d and a 15% to 20% HRR as described by Mulder et al. (Mulder et al., 2004). The dose was adjusted weekly on the basis of body weight and food intake. The amount of ivabradine in the diet was controlled monthly.

Electrocardiographic monitoring

Two months after aortic banding, 24 conscious rats (12 rats receiving ivabradine and 12 controls) underwent a 12-hour continuous-lead II electrocardiographic recording to assess the heart rate. Under light isoflurane anaesthesia, the rats were implanted with a transmitter and electrodes (TA 10ETA-F20, Data Sciences International) placed under the abdominal skin. The animals were housed individually and given the same treatment regimen described above. The ECG signal was recorded during the dark period of the standardized light/dark

cycle and stored for off-line analysis (PowerLab/16SP, AD Instruments). The mean heart rate was calculated and ventricular arrhythmias were identified and quantified manually.

Echocardiographic studies

Rats underwent echocardiography under isoflurane anaesthesia (1.0%-1.5% in oxygen), just before and then after 30 and 90 days of ivabradine treatment, using a Toshiba PowerVision 6000, SSA 370A apparatus equipped with a phase array 7.5-MHz transducer. The left ventricle was imaged as previously described (Prunier et al., 2002; Chen et al., 2004). Data were transferred to a computer (Ultrasound Image Workstation-300A, Toshiba) for off-line analysis. The following parameters were measured or calculated: end-diastolic and end-systolic LV diameters (LVEDD and LVESD, respectively); thickness of the interventricular septum and posterior wall; LV shortening fraction ((LVEDD – LVESD)/LVEDD); stroke volume (SV) as aortic VTI $\times$ [$\pi \times$ (diameter of LV outflow)^2/4], where VTI is the velocity-time integral; LV ejection fraction (SV/LVEDD^3); cardiac output (SV $\times$ HR); and peak velocity of early diastolic mitral filling (E), E-wave deceleration time and isovolumic relaxation time. Peak velocities of systolic (Sa) and early diastolic (Ea) LV motion were measured by pulsed-wave TDI, with the sample volume placed at the lateral corner of the mitral annulus, to assess global LV function in the longitudinal axis. E/Ea was calculated to estimate LV end-diastolic pressure ({Prunier et al., 2002; Slama et al., 2005). The strain rate of the LV posterolateral wall was derived from time-movement colour TDI and was measured in the parasternal short-axis view, as previously described (Chen et al., 2004).

Hemodynamic measurements

The hemodynamic study was performed after the last echographic examination, just before euthanasia (after 90 days of treatment) as previously described (Prunier et al., 2002; Chen et al., 2004). In brief, rats were sedated with ketamine (50 mg/g) and xylazine (10 mg/g)

and were ventilated. After left thoracotomy, a 2F micromanometer-tipped catheter (Millar, Houston, TX) was placed in the LV cavity through the apex. The LV pressure (peak systolic, end-diastolic and developed pressures), and its maximal and minimal derivatives (dP/dt_{max} and dP/dt_{min}) were recorded with a MacLab software. All data are reported as the means for 15 steady-state cardiac cycles.

Cardiac morphometry and molecular remodeling

Before and after the hemodynamic measurements, the rats were carefully inspected for pleural and abdominal effusion. Then, blood was removed by aortic puncture, and organs were immediately rinsed by infusion with cold saline. The lungs, atria and right and left ventricles were dissected and weighed. Then, cross-sections of the LV were made at the level of the papillary muscles, frozen in liquid nitrogen and stored at -80°C for analysis.

Cardiac fibrosis was assessed by semi-quantitative measurement of cardiac collagen density. Eight-µm-thick LV cryosections were stained with Sirius red and analysed microscopically (x500) by a trained operator blinded to the treatment arm. Fibrosis was scored from 0 to 9 as follows: 0-3 for expansion across the whole LV section, 0-3 for transmural expansion, 0 or 1 for myocytes necrosis, and 0 or 1 for perivascular fibrosis.

Frozen LV tissue samples were homogenized in ice-cold buffer, and total creatinine kinase, cytochrome c oxidase and citrate synthase activities were assayed after extraction, by using coupled enzyme systems as previously described (De Sousa et al., 1999). Other samples were used for mRNA quantification of type 2a sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), phospholamban (PLB), type 2 ryanodine receptor (RyR2) and atrial natriuretic peptide (ANP). After reverse transcription with random primers, the level of mRNA expression was assessed by real-time RT-PCR using a Light-Cycler device and the FastStart DNA Master SYBR Green Kit (Roche Diagnostics, Meylan, France). For quantification, a standard curve was generated with six different amounts of cDNA. The following oligo

primers were used: SERCA2a, 5'-ATGGACGAGACGCTCAAGTT-3' and 5'-CAAACTGTACAGGGCCAAT-3'; PLB, 5'-TGTGACGATCACAGAAGCC-3' and 5'-GCAGCAGACATATCAAGATGAG-3'; RyR2, 5'-GTGTTTGGATCCTCTGCAGTTCAT-3' and 5'-AGAGGCACAAAGAGGAATTCGG-3'; and ANP, 5'-CCTGCTAGACCACCTGGAG-3' and 5'-GGATCTTTTGCGATCTGCT-3'. Messenger RNA expression was normalized to that of the housekeeping gene ß-actin, amplified with primers 5'-TGGAATCCTGTGGCATCCATGAAAC-3' and 5'-TAAAACGCAGCTCAGTAACAGTCCG-3'.

Statistical analysis

Continuous data are expressed as means ± SEM and categorical data as percentages. Comparison of means among groups was based on ANOVA and the Newman-Keuls test. Group-to-group comparisons were done using the Bonferroni correction. Linear regression analysis was based on the least-squares method. $P<0.05$ was considered to denote a significant difference.

Results

Level of ivabradine-induced HRR

Twelve-hour ECG monitoring revealed a 19% HRR in rats with aortic banding treated with ivabradine as compared with untreated rats (277±20 vs 345±26 beats/min, $p<0.005$), throughout the 12-hour recording period (Fig. 1). A similar 19% HRR in ivabradine-treated as compared with untreated rats was observed during echography (Table 1).

Effect of aortic banding and HRR on LV hypertrophy

Echographic examination revealed a marked LV hypertrophy as early as 14 days after aortic banding (i.e. before ivabradine treatment) in the two banded groups (Figure 2). At sacrifice, LV hypertrophy in the untreated group averaged 55% (Table 2).

Ivabradine treatment was associated with more marked LV hypertrophy (70%, +10% vs untreated rats, $p<0.05$), but not with an increase in LV diastolic diameter (11.5±0.1 vs 11.1±0.2 mm, $p=0.09$, Table 1).

Effect of aortic banding and HRR on LV systolic function

Aortic banding was associated with a gradual decrease in LV stroke volume, LV shortening fraction, and cardiac output that averaged respectively 41%, 34% and 38% at 3 months (Fig. 2). TDI parameters (Sa peak velocity and systolic peak strain rate) were also reduced, whereas dP/dt_{max} was unchanged (Table 1). HRR was associated with a 19% increase in stroke volume ($p<0.01$) but no change in cardiac output or in other parameters of LV systolic function. As HRR was associated with a proportional increase in the ejection time, the stroke volume/ejection time ratio did not change.

Effect of aortic banding and HRR on LV diastolic function and filling pressure

Aortic banding was associated with a gradual alteration of LV filling, as shown by an increased E/Ea at echo and by hemodynamic data at 3 months, characterized by a marked

increase in LV filling pressure (Fig. 2, Table 1). The increased E/Ea and almost unchanged LV end-diastolic volume suggested a decrease in LV compliance in banded rats compared to sham-operated controls.

In ivabradine-treated rats, LV filling pressure increased further, as shown by increased E/Ea and LVEDP values as compared with untreated rats. Increased atrial weight, lung weight and RV weight in rats treated with ivabradine for 3 months (38%, 30% and 54%, respectively vs untreated rats, $p<0.01$) were consistent with increased LV filling pressure This was further supported by the increased prevalence of pleural or peritoneal effusion in these rats (36 vs 19%, $p<0.05$, Table 2).

Effect of aortic banding and HRR on tissue and molecular LV remodeling

Marked LV perivascular and subendocardial fibrosis was seen in banded rats at 3 months. This fibrosis further increased in ivabradine-treated rats (fibrosis score: 6.4±0.3 vs 5.2±0.2 in treated and untreated rats, respectively, $p < 0.05$) and was essentially located in the subendocardium (Fig. 3).

The ANP mRNA level increased markedly in the LV of banded rats as compared to sham-operated rats (Table 3). A further increase was observed in ivabradine-treated rats (6.4±0.3 vs 5.2±0.2 a.u. in treated and untreated rats, respectively, $p<0.05$). A non-significant increase in SERCA2a mRNA levels was found in banded rats compared to sham-operated rats. In contrast, SERCA2a mRNA levels decreased in ivabradine-treated rats as compared to untreated banded rats (1.05±0.09 vs 1.42±0.13, $p<0.05$). Citrate synthase and cytochrome oxidase activities did not differ in the three rat groups. In contrast, creatine kinase activity fell in the two banded groups as compared to sham-operated controls ($p<0.05$ for the two comparisons), with no difference between ivabradine-treated and untreated rats.

Effect of treatment cessation at the end of the study

After 3 months, treatment with ivabradine was stopped for 3 days in a subgroup of 8 rats, and echographic analyses were repeated after ivabradine wash-out. On treatment cessation, the heart rate returned to the level of untreated rats, LV stroke volume decreased, and cardiac output was unchanged (table 4). In addition, E/Ea and LV end-diastolic diameter decreased. Taken together, these data indicated that some HRR-related effects were rapidly reversible.

Discussion

This study demonstrates that pressure overload-induced heart failure in rats is not improved by chronic HRR. This model has been thoroughly characterized in a number of previous studies. After banding of the ascending aorta, the pressure overload of the left ventricle increases gradually during rat growth, resulting in progressive LV hypertrophy, LV fibrosis and congestive heart failure (Feldman et al., 1993; Litwin et al., 1995). A number of studies have shown beneficial effects of angiotensin-converting-enzyme inhibitors, beta-blockers and statins in aortic banding-induced heart failure (Weinberg et al., 1994; Kagaya et al., 1996; Marano et al., 2002; Pape et al., 2002; Grimm et al., 2001; Indolfi et al., 2002). Several reasons led us to postulate that HRR itself could also be beneficial. First, hypertrophy impairs relaxation, which shortens the duration of LV filling (Leite-Moreira et al., 1999). Accordingly, we postulated that HRR would improve LV filling. In addition, ivabradine-mediated HRR has no negative lusitropic effect and further increases the LV filling duration as compared to beta-blockers (Colin et al., 2002). Second, as the force-frequency relationship is altered in heart failure (Mulieri et al., 1992), we suspected that HRR might allow the heart to operate on the ascending limb of this relation. Third, myocardial perfusion, which is altered in hypertrophied ventricles (Hittinger et al., 1995), occurs mainly during diastole. Accordingly, we postulated that HRR, by extending the diastole, would improve myocardial perfusion and, thus, myocardial metabolism. In addition, angiogenic effects as well as an increase in coronary reserve were demonstrated using alinidine-mediated HRR (Lei et al, 2004). Finally, because the heart rate is a major determinant of myocardial O_2 consumption (Colin et al., 2003), we thought that HRR could be beneficial in heart failure, as in coronary artery disease (Shinke et al, 1999; Borer et al., 2003).

In our model, chronic HRR increased LV filling pressure, as demonstrated by echographic and hemodynamic findings and by a marked increase in atrium and lung weight.

An HRR-induced worsening of LV diastolic dysfunction was suggested by a markedly increased LVEDP, with no significant increase in LV end-diastolic volume. Such an effect might be explained as follows. First, HRR per se increased LV filling but aortic banding physically precluded any resulting increase in LV stroke volume and therefore led to a major rise in LVEDP because of the poor LV compliance characteristic of pressure-overload-induced LVH. Second, elevated LVEDP further increased LV hypertrophy and fibrosis, which further reduced LV compliance. In this type of maladaptive LV hypertrophy, the main mechanism leading to LV hypertrophy and fibrosis is considered to be the increase in wall stress, although neuro-humoral alterations are likely also to play a role (Mercadier et al., 2007). Regarding LV systolic wall stress, we could not directly measure the systolic pressure gradient through the aortic clip. However, the mean pressure gradient would likely have been little altered by HRR, as the stroke volume/ejection time ratio did not change (Dangas et al., 1997). The HRR-related increase in LV ejection duration clearly increased the duration of systolic LV wall stress, a phenomenon that might worsen LV hypertrophy. LV diastolic wall stress also was most likely increased by HHR, because of the marked increase in both LV filling pressure and filling duration, associated with a slight increase in LV volume. In addition, hypertrophy, as well as high diastolic and systolic LV wall stress, favours myocardial ischemia and subsequent fibrosis, especially in the subendocardium (Hittinger et al., 1989).

To determine whether the HRR-associated structural and functional changes observed were reversible, we withdrew ivabradine treatment in 8 rats. Three days after treatment cessation the heart rate returned to the values observed in untreated rats, while LV diastolic diameter fell and a major decrease in E/Ea occurred, indicating a marked decrease in LV filling pressure. These results, which are consistent with a downward shift of the operating point along the same diastolic pressure-volume relationship, strongly suggest that HRR was

directly responsible for the high LV filling pressure in these ventricles characterised by low compliance.

Regarding LV systolic function, pressure overload resulted in a depressed LV ejection fraction, which is related more to afterload mismatch than to decreased LV contractility (Ross et al., 1976). Indeed, LV fractional shortening was markedly decreased while LV dP/dt_{max}, which is less load-dependent than the ejectional parameter, was unchanged. Importantly, HRR was not associated with significant changes in isovolumic or ejectional parameters of LV systolic function, in keeping with ivabradine's lack of a negative inotropic effect. At the subcellular level, however, HRR was associated with increased ANP mRNA and decreased SERCA2a mRNA levels as compared with untreated rats, and this latter decrease has been shown to correlate with the transition to cardiac failure in this model (Feldman et al., 1993). As previously reported (De Sousa et al., 1999), aortic banding resulted in decreased activity of creatine kinase, an enzyme involved in energy transfer within cardiac myocytes. No further decrease in this activity was observed in ivabradine-treated rats, at a time when both LV hypertrophy and dysfunction were more severe than in untreated rats, indicating some degree of preservation of energy metabolism by HRR.

Previous studies have shown beneficial effects of I*f* current inhibitors in the myocardial infarction-induced heart failure model. Mulder et al. (Mulder et al., 2004) demonstrated that a 16% ivabradine-mediated HRR maintained for 90 days increased the LV shortening fraction without LV dilation, and that this effect persisted after treatment cessation. Using isolated Langendorff-perfused hearts, authors also observed a leftward shift of the developed pressure-volume relationship in ivabradine-treated rats as compared with controls. Interestingly, this was associated with a decrease in collagen density and in the plasma noradrenaline concentration. Recently, Dedkov et al showed that a 25% ivabradine-induced HRR after myocardial infarction improved maximal myocardial perfusion, as well as the

coronary reserve, an effect which was related to reduced periarteriolar collagen content (Dedkov et al., 2007). Using an 8-week 25% zatebradine-mediated HRR, Hu el al observed mitigated results. In large myocardial infarctions, HRR enhanced LV performance with no change in LV remodeling. In contrast in small myocardial infarctions, HRR favoured LV dilation and increased LV filling pressures (Hu et al, 2004). The difference between our results and those obtained in other models may be explained by different patterns of LV remodelling. Indeed, post-myocardial infarction remodelling is characterised by eccentric LV hypertrophy, a lesser decrease in LV chamber compliance (Pfeffer et al., 1991; Hu et al, 2004), and a profound decrease in LV systolic function. Moreover, it is likely that HRR also alters ventriculo-arterial coupling. In heart failure, HRR has been shown to improve ventriculo-arterial coupling, in part by decreasing arterial elastance (Yamakawa et al., 1996; Albaladejo et al., 2003). Our model of fixed aortic banding may not permit HRR to alter arterial elastance, precluding one possible benefit of HRR. Regarding myocardial O_2 consumption, potential HRR-related benefits may have been blunted in our model because myocardial O_2 consumption is poorly determined by the heart rate when afterload is high (Nagatsu et al., 2000). Finally, beta-blockers could be more effective than pure bradycardic agents in this model, because of their negative inotropic effect and the subsequent decrease in LV load. In this respect, although some studies have shown positive effects (Marano et al., 2002; Pape et al., 2002; Grimm et al., 2001), the impact of beta-blocker-induced HRR on LV hypertrophy remains uncertain (Marano et al., 2002). Altogether, our results suggest that HRR itself may not be beneficial in all types of heart failure. If HRR is beneficial in heart failure associated with LV dilation, such as ischemic and dilated cardiomyopathy, it may not be so in case of aortic stenosis and hypertrophic cardiomyopathy associated with a major decrease in LV compliance. In this latter clinical setting, prolonged diastolic filling induced by HRR might also greatly increase LV filling pressure in stiff ventricles that are less prone to dilate.

Moreover, impaired sinus node function and arrhythmogenic ion-channel remodelling during heart failure needs also to be taken into account (Nattel et al., 2007).

Our study has some limitations. Our measurement of LVEDP *in vivo* clearly yielded underestimated values due to the direct LV puncture in open-chest rats. LVEDP indirect assessment using Doppler E/Ea probably better reflected actual pressures. Because we tested only one dosage of ivabradine, the effects of other dosages are unknown. Similarly, we cannot directly extrapolate the results of our study to situations in which ivabradine treatment starts later after aortic banding.

In summary, long-term HRR showed no beneficial effects in pressure overload-induced LV hypertrophy in the rat. On the contrary, it favoured LV hypertrophy, fibrosis, diastolic pressure increases and their congestive consequences. Taken together, these results indicate that pure HHR should not be considered as a pertinent therapeutic approach in patients unable to increase their stroke volume because of severe LV outflow track obstruction and/or patients with severe alteration in LV compliance. Additional studies of HRR produced by different dosages of ivabradine or other pharmacologic agents in different models of cardiac diseases are needed to refine the conditions in which pure HRR exerts its most beneficial effects.

Acknowledgements

We thank Dominique Fortin for helpful technical assistance.

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Footnotes

This work was supported in part by a grant from the Institut de Recherches Internationales Servier, Courbevoie, France.

Jean-Jacques Mercadier and Michèle Heimburger are supported by Université Paris Diderot, Inserm and EU FP6 grant LSHM-CT-2005-018833, EUGeneHeart.

Legends for Figures

Figure 1: 12-h Holter recording of mean heart rate in banded rats, 2 months after aortic banding (◆, untreated group, □ , ivabradine treated group).

Figure 2: Serial echographic examinations in banded and sham-operated rats just before treatment onset (two weeks after surgery), and after 1 and 3 months of treatment (□, sham-operated rats, ▒ , untreated banded rats, ■ **,** ivabradine-treated banded rats).

Figure 3: Representative LV tissue samples with Sirius red staining. A, sham-operated rats; B, 3-month banded rats; arrows indicate fibrosis.

Table 1: Echographic and hemodynamic values at 3 months in sham-operated rats and in aortic-banded rats treated or not treated with ivabradine, 10 mg/kg/d. LV: left ventricular; LVEDD: LV end-diastolic diameter; BW: body weight; IVS: interventricular septum; E: early diastolic transmitral maximal velocity; Ea: early diastolic maximal velocity at the mitral annulus; IVRT: isovolumic relaxation time; LVEDP: LV end diastolic pressure. * $p < 0.05$, ** $p < 0.01$, vs untreated rats.

	Shams (n = 12-16)	**Aortic-banded rats**	
		Untreated (n = 17-27)	**Ivabradine, 10 mg/kg/d** (n = 16-29)
Heart rate (beats/min)	330 ± 7	347 ± 6	281 ± 6 **
LVEDD/BW (mm/kg)	21.8 ± 0.6 **	24.2 ± 0.5	25.6 ± 0.7
IVS thickness (mm)	0.96 ± 0.03 **	1.86 ± 0.03	1.93 ± 0.03
IVS thickness/LVEDD	0.18 ± 0.01 **	0.33 ± 0.01	0.33 ± 0.01
Cardiac output (ml/min)	216 ± 6 **	134 ± 4	127 ± 5
Stroke volume/BW (µL/g)	1.27 ± 0.05 **	0.83 ± 0.02	1.06 ± 0.03 **
Ejection time (msec)	76 ± 6 *	89 ± 4	98 ± 6 *
Stroke volume/ejection time	8.6 ± 0.7 *	4.3 ± 0.6	4.5 ± 0.8
LV shortening fraction (%)	41 ± 1 **	27 ± 1	27 ± 1
Sa (cm/s)	4.61 ± 0.14 **	2.79 ± 0.10	2.68 ± 0.09
strain rate (sec^{-1})	11.61 ± 0.41 *	8.35 ± 0.32	7.43 ± 0.21
E/Ea	15 ± 1 **	23 ± 1	31 ± 2 **
IVRT (ms)	22.0 ± 0.7 **	14.5 ± 0.8	11.6 ± 0.6 **
Systolic LVP (mmHg)	119 ± 6 *	203 ± 9	226 ± 22
LVEDP (mmHg)	1.8 ± 0.4 *	3.2 ± 0.4	7.8 ± 1.2 **
dP/dt_{max} ($mmHg.sec^{-1}$)	7595 ± 120	8309 ± 102	7831 ± 319
dP/dt_{min} ($mmHg.sec^{-1}$)	6280 ± 243	6420 ± 110	6281 ± 200

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Table 2: Morphometric values at 3 months in sham-operated rats and aortic-banded rats treated or not treated with ivabradine, 10 mg/kg/d. HRR: heart rate reduction; LV: left ventricular; RV: right ventricular; BW: body weight. * $p<0.05$, ** $p<0.01$, versus untreated aortic-banded rats.

	Shams (n = 14)	**Aortic-banded rats**	
		Untreated (n = 26)	**Ivabradine, 10 mg/kg/d (n =27)**
Body weight (g)	542 ± 14 *	493 ± 11	483 ± 12
LV weight (g)	1134 ± 29 **	1615 ± 36	1721 ± 33 *
LV weight / BW (g/g)	2.12 ± 0.05 **	3.28 ± 0.09	3.60 ± 0.09 *
RV weight /BW (g/g)	0.57 ± 0.03 *	0.71 ± 0.08	1.09 ± 0.07 **
Atrial weight /BW (g/g)	0.25 ± 0.02 **	0.74 ± 0.08	1.03 ± 0.07 **
Lung weight / BW (g/g)	3.43 ± 0.08	4.95 ± 0.55	6.84 ± 0.45 *
Presence of pleural or peritoneal effusion	0 **	19%	36% *

Table 3: Histologic and molecular results at 3 months in sham-operated rats and aortic-banded rats treated or not treated with ivabradine, 10 mg/kg/d. HRR: heart rate reduction; ANP: atrial natriuretic peptide; SERCA: sarco-endoplasmic reticulum Ca^{2+}-ATPase; RyR2: type 2 ryanodine receptor. mRNA levels are given in arbitrary units. * $p<0.05$, ** $p<0.01$, vs untreated animals.

	Shams (n = 6-9)	**Aortic-banded rats**	
		Untreated (n = 8-15)	**Ivabradine, 10 mg/kg/d (n = 10-15)**
LV fibrosis score (0 to 9)	0 **	5.2 ± 0.2	6.4 ± 0.3 *
Atrial natriuretic peptide mRNA level	0.08 ± 0.02 *	1.23 ± 0.16	1.88 ± 0.19 **
SERCA2a mRNA level	1.23 ± 0.10	1.42 ± 0.13	1.05 ± 0.09 *
RyR2 mRNA level	1.09 ± 0.24	0.83 ± 0.09	0.71 ± 0.13
Phospholamban mRNA level	0.97 ± 0.11	0.92 ± 0.09	0.85 ± 0.08
Citrate synthase (IU/g prot)	730 ± 66	631 ± 42	528 ± 46
Cytochrome c oxidase (IU/g prot)	846 ± 110	793 ± 58	783 ± 102
Creatine kinase (IU/g prot)	4387 ± 248 *	3162 ± 333	3218 ± 301

Table 4: Echographic values before and 3 days after cessation of 3 months of treatment with ivabradine, 10 mg/kg/d, in 8 rats. * $p<0.05$ and ** $p<0.01$, vs treatment.

	On treatment	**3 days after treatment cessation**
Heart rate (beats/min)	283 ± 14	315 ± 7 **
LVEDV (mm)	11.8 ± 0.4	11.6 ± 0.4 *
LV shortening fraction (%)	33 ± 4	33 ± 6
Stroke volume (µL)	516 ± 14	490 ± 18 *
Cardiac output (ml/min)	144 ± 7	155 ± 5
E/Ea ratio	32 ± 2	23 ± 2 *

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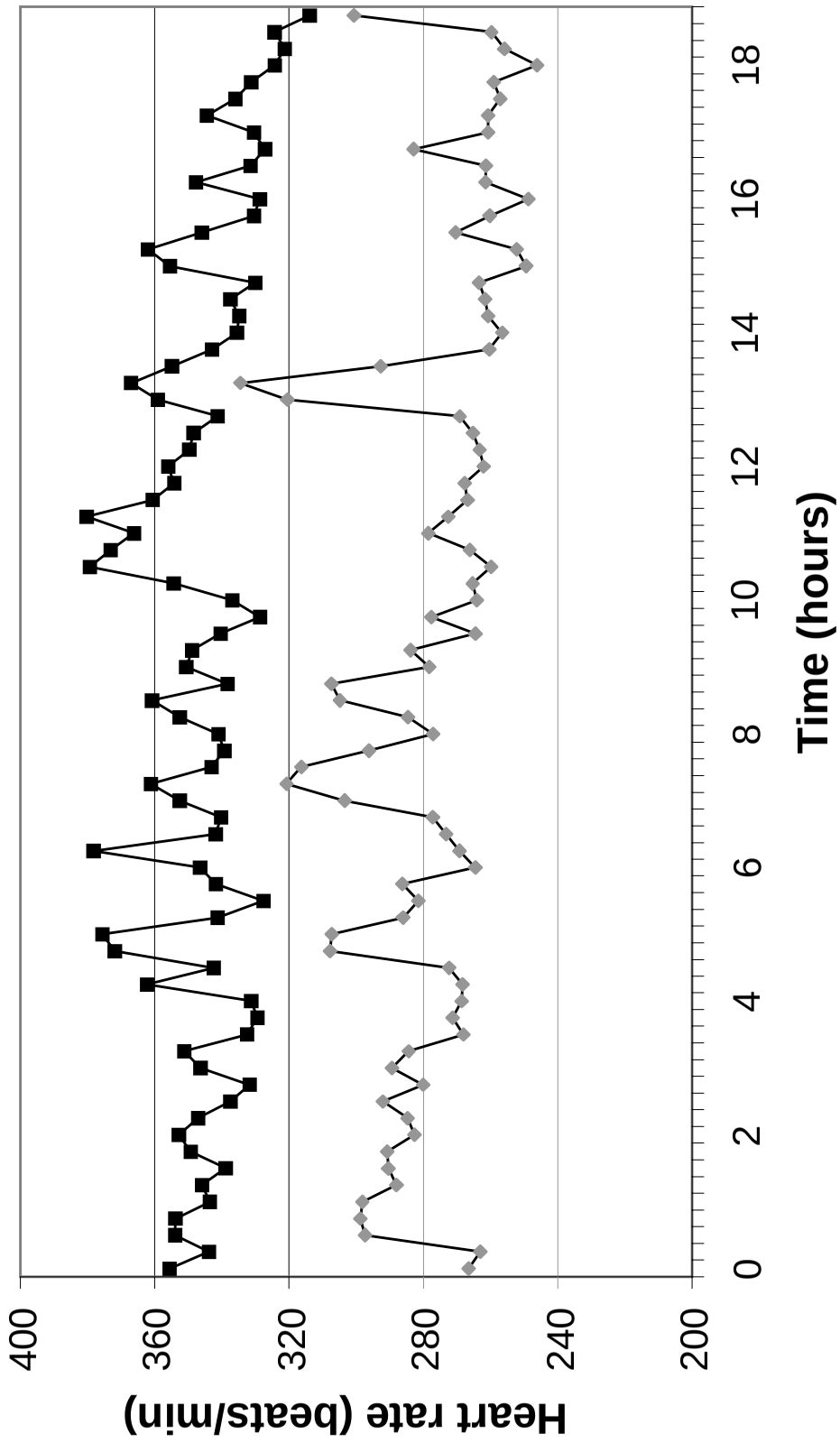

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Figure 2

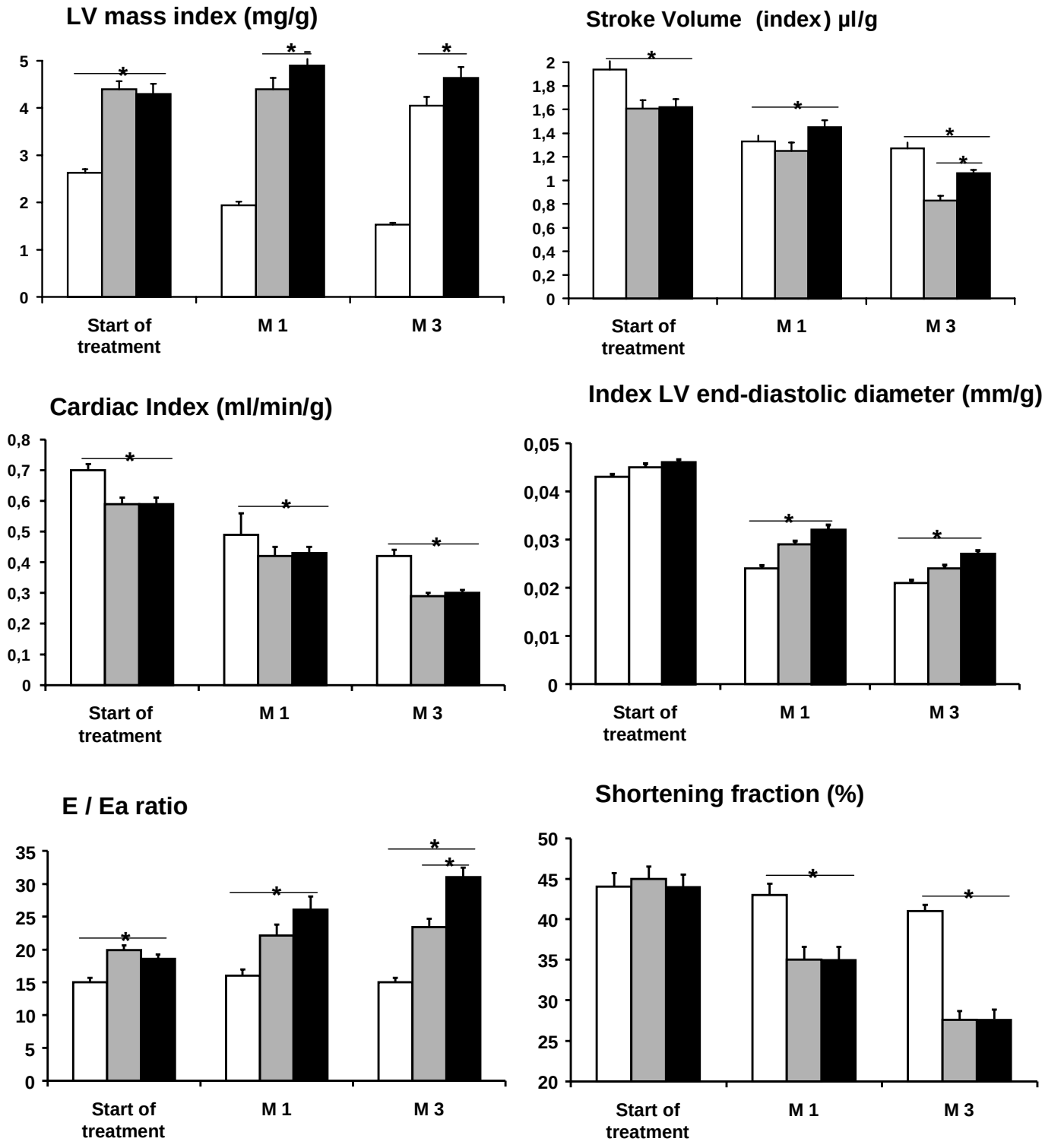

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Figure 3A

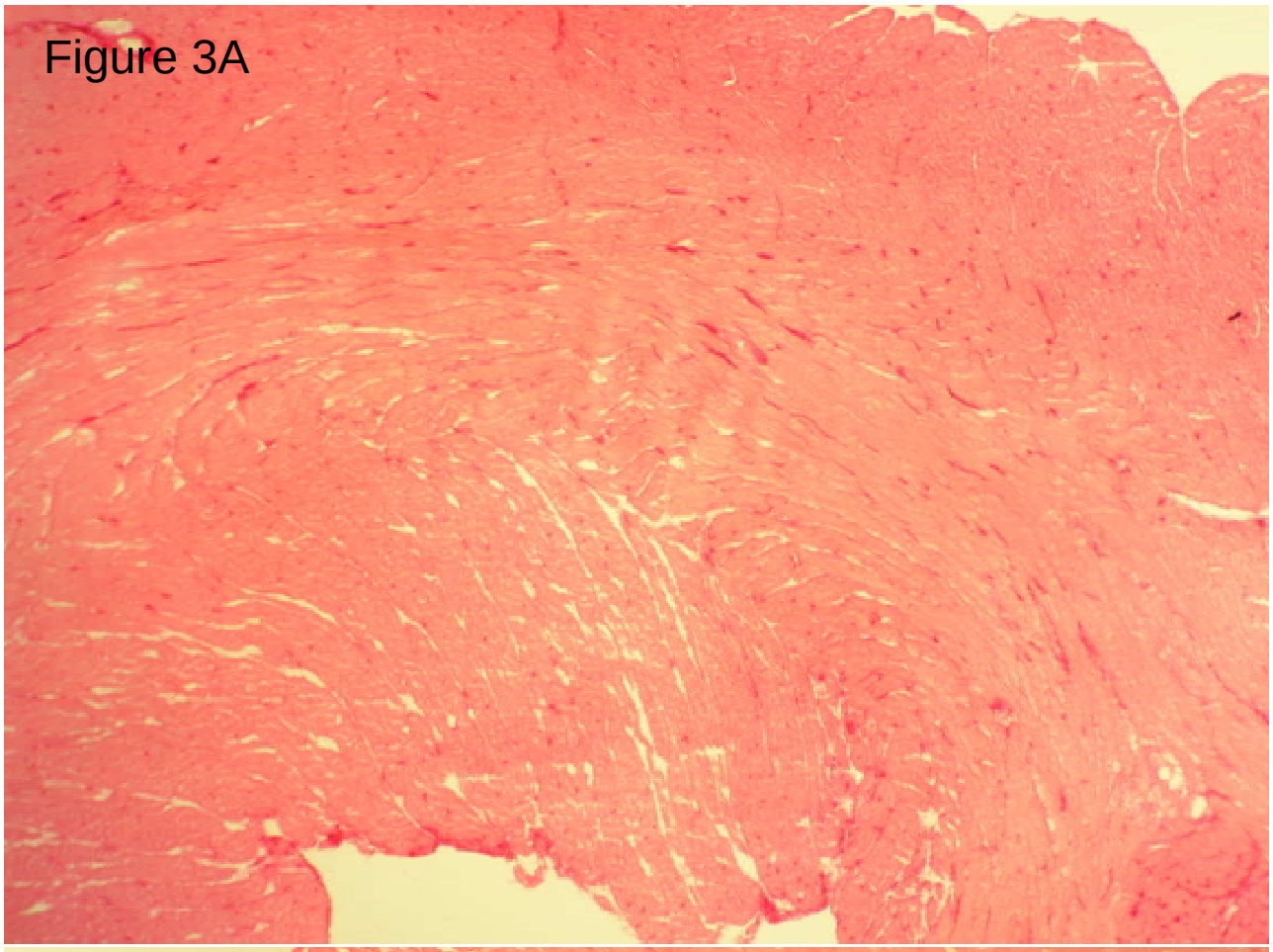

Figure 3B