

# Long-Term Heart Rate Reduction Induced by the Selective $I_f$ Current Inhibitor Ivabradine Improves Left Ventricular Function and Intrinsic Myocardial Structure in Congestive Heart Failure

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**Background**—Heart rate reduction (HRR) improves left ventricular (LV) filling, increases myocardial  $O_2$  supply, and reduces myocardial  $O_2$  consumption, which are all beneficial in congestive heart failure (CHF). However, the long-term effects of HRR on cardiac function and remodeling are unknown.

**Methods and Results**—We assessed, in rats with CHF, the effects of long-term HRR induced by the selective  $I_f$  current inhibitor ivabradine (as food admix for 90 days starting 7 days after coronary artery ligation). To assess intrinsic modifications of LV tissue induced by long-term HRR, all parameters were reassessed 3 days after interruption of treatment. Ivabradine decreased heart rate over the 90-day treatment period ( $-18\%$  versus untreated at  $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ), without modifying blood pressure, LV end-diastolic pressure, or  $dP/dt_{\text{max/min}}$ . Ivabradine significantly reduced LV end-systolic but not end-diastolic diameter, which resulted in preserved cardiac output due to increased stroke volume. In the Langendorff preparation, ivabradine shifted LV systolic but not end-diastolic pressure-volume relations to the left. Ivabradine decreased LV collagen density and increased LV capillary density without modifying LV weight. Three days after interruption of treatment, the effects of ivabradine on LV geometry, shortening, and stroke volume persisted despite normalization of heart rate.

**Conclusions**—In rats with CHF, long-term HRR induced by the selective  $I_f$  inhibitor ivabradine improves LV function and increases stroke volume, preserving cardiac output despite the HRR. The improvement of cardiac function is related not only to the HRR per se but also to modifications in the extracellular matrix and/or function of myocytes as a consequence of long-term HRR. (*Circulation*. 2004;109:1674-1679.)

**Key Words:** heart failure ■ heart rate ■ drugs ■ pathology

Enhanced heart rate, which is generally observed in patients with congestive heart failure (CHF), attenuates the decrease in cardiac output or preserves cardiac output at the cost of impaired left ventricular (LV) filling, increased myocardial  $O_2$  consumption, and reduced coronary perfusion time. Moreover, these phenomena are involved in the development of the LV dysfunction and/or CHF observed after persistent tachycardia.<sup>1,2</sup>

Thus, heart rate reduction (HRR) should, in theory, be beneficial in CHF. Indeed, large-scale clinical trials conducted with  $\beta$ -blockers in CHF suggest such an essential role for HRR, because the effects of  $\beta$ -blockers on cardiac function and survival are correlated with the magnitude of the HRR.<sup>3-5</sup> Furthermore, pacing abolishes the increase of LV contractile function in a dog model of CHF after long-term  $\beta$ -blocker treatment.<sup>6</sup> Although these data support the hypothesis of an essential role of HRR in the effects observed after long-term

$\beta$ -blockade, other mechanisms, such as prevention of  $\beta$ -receptor downregulation or direct myocardial damage caused by catecholamines,<sup>7</sup> are potentially involved and cannot be excluded. Thus, whether long-term selective HRR per se exerts beneficial effects on cardiac function in CHF is still unclear.

Ivabradine (S16257-2) is a novel selective inhibitor of cardiac pacemaker  $I_f$  current, or sinoatrial node modulators,<sup>8</sup> which induces in humans and animals a selective and dose-dependent HRR<sup>9-11</sup> without modifying atrioventricular or intraventricular conduction or contractility.<sup>12</sup> We used this specific heart rate-lowering agent to investigate, in a rat model of CHF, the effects of long-term “pure” HRR on LV function and remodeling.

## Methods

The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

Received March 31, 2003; de novo received July 15, 2003; revision received November 14, 2003; accepted November 17, 2003.

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*Circulation* is available at <http://www.circulationaha.org>

DOI: 10.1161/01.CIR.0000118464.48959.1C

## Animals and Treatments

Myocardial infarction was produced in 11-week-old male Wistar rats (Charles River, France) by left coronary artery ligation, according to the method of Pfeffer et al<sup>13</sup> and modified in our laboratory.<sup>14</sup> Twelve rats were subjected to the same protocol except that the snare was not tied, and these rats served as the sham group. All rats were allowed standard rat chow and drinking water ad libitum and were maintained on a 12-hour/12-hour light/dark cycle. Seven days after ligation, infarcted rats were randomized to receive either placebo or ivabradine given as food additive for 90 days at a dose of 0.3, 1, 3, or 10 mg · kg<sup>-1</sup> · d<sup>-1</sup> (n=24 per group).

## Hemodynamic Measurements

Systolic blood pressure and heart rate were determined (plethysmography) in conscious rats just before the start and after 4, 30, and 90 days of treatment. At the end of the 90-day treatment, the rats were anesthetized with pentobarbital (50 mg/kg IP). The right carotid artery was cannulated with a micromanometer-tipped catheter (Millar Instruments) and advanced into the LV for recording of LV pressures and its maximal and minimal rate of rise (dP/dt<sub>max</sub> and dP/dt<sub>min</sub>).

## Echocardiographic Studies

Transthoracic Doppler echocardiographic studies were performed with an echocardiographic system (HDI 5000, ATL) equipped with an 8- to 5-MHz transducer in rats anesthetized with methohexital (50 mg/kg sodium IP) just before the start and after 30 and 90 days of treatment, as described previously.<sup>14</sup> Posterior end-diastolic and end-systolic LV posterior wall thicknesses and diameters were measured by the American Society of Echocardiology leading-edge method. In addition, LV outflow velocity was measured by pulsed-wave Doppler, and cardiac output (CO) was calculated as CO=aortic VTI × [π × (LV outflow diameter/2)<sup>2</sup>] × heart rate, where VTI is the velocity-time integral.

## Isolated Heart Preparation

In vitro LV function was determined in randomly selected CHF rats, either untreated or treated with ivabradine at a dose of 10 mg · kg<sup>-1</sup> · d<sup>-1</sup>.<sup>15</sup> In brief, after anesthesia, the heart was excised and placed in ice-cold Krebs-Henseleit solution. Within 30 seconds, the heart was transferred to a Langendorff apparatus, perfused at constant hydrostatic pressure (80 cm H<sub>2</sub>O), and paced (250 bpm). A balloon was inserted into the LV and connected to a pressure transducer to record LV pressure and dP/dt<sub>min/max</sub>. After 30 minutes of perfusion, baseline LV pressures (0 mm Hg end-diastolic pressure) and responses to standard increases of end-diastolic volume were recorded up to a maximal LV end-diastolic pressure of 40 mm Hg.

## Modifications of the Intrinsic Properties of LV Tissue

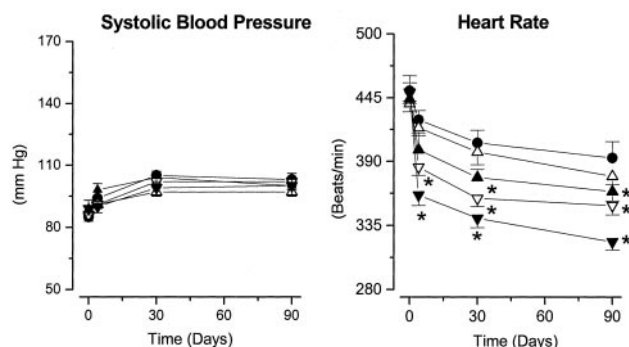
To assess intrinsic modifications of LV tissue properties induced by long-term HRR, hemodynamic and echocardiographic parameters and in vitro LV function were reassessed 3 days after interruption of the 3-month treatment in a small, randomly selected number of animals from the group treated with 10 mg · kg<sup>-1</sup> · d<sup>-1</sup> ivabradine.

## Plasma Catecholamines

After determination of cardiac hemodynamics, an arterial blood sample was taken and centrifuged at 3000g. The plasma was stored at -80°C for subsequent determination of noradrenaline concentration by high-performance liquid chromatography and electrochemical detection.

## Cardiac Morphometry

Morphometric analyses were performed as described previously.<sup>14</sup> The atria and ventricles were weighed, and a section of the LV, at the level of the papillary muscles, was immersed in Bouin fixative solution. After fixation, the sections were dehydrated and embedded in paraffin, and 5-μm-thick slices of each section were obtained and stained with Sirius red.



**Figure 1.** Systolic blood pressure and heart rate measured in CHF rats, either untreated (●) or treated by ivabradine at the dose of 0.3 (△), 1 (▲), 3 (▽), or 10 (▼) mg · kg<sup>-1</sup> · d<sup>-1</sup> (n=8 to 24 per group). \**P*<0.05 vs untreated CHF.

Infarct size was determined with image-analysis software (Cyber-view, Cervus International) coupled to a video microscope. Infarct size was calculated as (endocardial+epicardial circumference of the infarcted tissue)/(endocardial+epicardial circumference of the LV) and expressed as a percentage.

For the measurement of cardiac collagen density, slides were enlarged 500 times with a microscope connected to the same image-analysis system. Collagen density was calculated in the viable part of the LV as the surface occupied by collagen divided by the surface of the image. LV coronary capillary density was determined as previously described,<sup>16</sup> both in sham animals and in CHF rats, either untreated or treated with ivabradine at a dose of 10 mg · kg<sup>-1</sup> · d<sup>-1</sup>.

## Statistical Analysis

All results are given as mean±SEM. For echocardiographic parameters, values at day 0 (before treatment) are presented as the mean±SEM obtained for all infarcted animals, because homogeneity among groups has been established. Differences for parameters obtained at each time point were evaluated by *t* test or by 1-way ANOVA, followed by a Dunnett test in case of significance, with Syst Stat software. Differences were considered significant at the level of *P*<0.05.

## Results

### Infarct Size

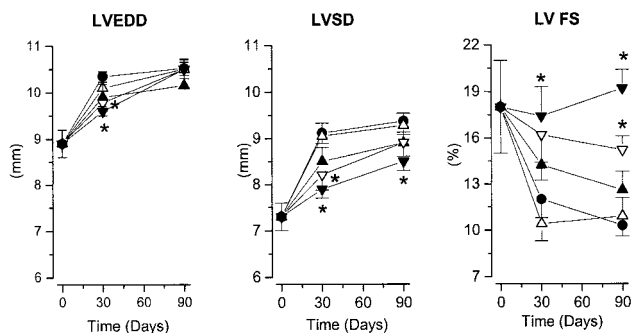
Mean infarct size in the groups treated with 0.3, 1, 3, and 10 mg · kg<sup>-1</sup> · d<sup>-1</sup> was similar (45±3%, 42±2%, 40±3%, and 42±2%, respectively) compared with the untreated group (41±3%).

### Systolic Blood Pressure and Heart Rate in Conscious Rat

Before the start of treatment, systolic blood pressure and heart rate were comparable among the groups of infarcted rats (Figure 1). Compared with untreated animals, ivabradine reduced heart rate significantly and dose dependently after 4 days of treatment (-2%, -6%, -10%, and -15% at 0.3, 1, 3, and 10 mg · kg<sup>-1</sup> · d<sup>-1</sup>, respectively). The reduction of heart rate remained stable throughout the study (-4%, -7%, -10%, and -18% at 0.3, 1, 3, and 10 mg · kg<sup>-1</sup> · d<sup>-1</sup>, respectively, after 3 months). Moreover, ivabradine never modified systolic blood pressure.

### LV Diameters and Fractional Shortening

Before the start of treatment, LV end-diastolic diameter (LVEDD), LV systolic diameter, and LV fractional shorten-



**Figure 2.** LV end-diastolic cavity diameter (LVEDD), LV systolic diameter (LVSD), and LV fractional shortening (LV FS) measured in anesthetized CHF rats, either untreated (●) or treated by ivabradine at the dose of 0.3 (△), 1 (▲), 3 (▽), or 10 (▼)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  ( $n=8$  to 19 per group). \* $P<0.05$  vs untreated CHF.

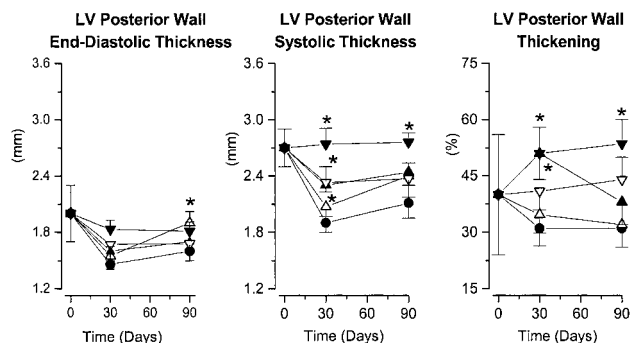
ing were comparable among the different groups of infarcted rats (Figure 2). Ivabradine slightly reduced LVEDD after 30 days of treatment at 3 and 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  but did not modify LVEDD after 90 days of treatment. In contrast, LV systolic diameter was dose-dependently reduced after 30 and 90 days of treatment, reaching statistical significance at doses of 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  after 90 days. Moreover, ivabradine, which did not per se increase LV fractional shortening, prevented, in a dose-dependent manner, the deterioration in LV fractional shortening observed with time in untreated CHF animals after 30 and 90 days (Figure 2).

### Posterior Wall Thickness and Thickening

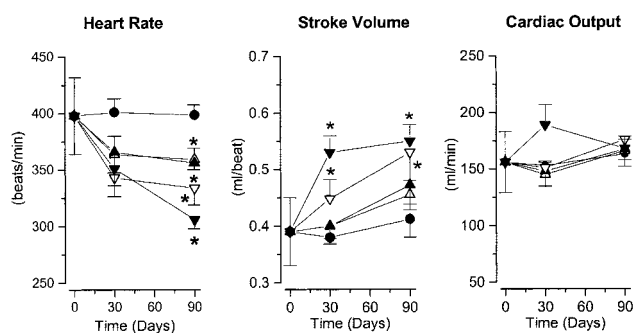
Before the start of treatment, LV posterior wall thickness and thickening were comparable among the groups of infarcted rats (Figure 3). Ivabradine modified LV end-diastolic wall thickness only modestly. In contrast, LV systolic wall thickness was further reduced in CHF rats over 90 days, and ivabradine limited or even prevented this worsening in a dose-related manner. Moreover, ivabradine dose-dependently improved LV posterior wall thickening after 30 and 90 days (Figure 3).

### Stroke Volume and Cardiac Output

Before the start of treatment, stroke volume and cardiac output were comparable among all infarcted rats (Figure 4).



**Figure 3.** LV posterior wall end-diastolic thickness, LV posterior wall systolic thickness, and LV posterior wall thickening measured in anesthetized CHF rats, either untreated (●) or treated by ivabradine at the dose of 0.3 (△), 1 (▲), 3 (▽), or 10 (▼)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  ( $n=8$  to 19 per group). \* $P<0.05$  vs untreated CHF.



**Figure 4.** Heart rate, stroke volume, and cardiac output measured in anesthetized CHF rats, either untreated (●) or treated by ivabradine at the dose of 0.3 (△), 1 (▲), 3 (▽), or 10 (▼)  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$  ( $n=8$  to 19 per group). \* $P<0.05$  vs untreated CHF.

In anesthetized rats, heart rate was dose-dependently reduced by ivabradine after both 30 and 90 days of treatment (Figure 4). Ivabradine dose-dependently increased stroke volume after both 30 and 90 days of treatment, reaching statistical significance at 3 and 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , which resulted in preservation of cardiac output throughout the study.

### Hemodynamic Parameters

Compared with untreated CHF rats, ivabradine did not significantly modify LV systolic pressure, LV  $\text{dP}/\text{dt}_{\text{max}}$ , or LV  $\text{dP}/\text{dt}_{\text{min}}$ , and it slightly but nonsignificantly reduced LV end-diastolic pressure in the 3- and 10-mg/d-treated group (Table 1).

### In Vitro LV Function

Compared with untreated CHF rats, the LV systolic pressure–volume relation was shifted to the left, the LV end-diastolic pressure–volume relation was not modified, and the LV developed pressure–volume relation was shifted upward by ivabradine (Figure 5).

### LV Morphology

After 90 days, LV weight and LV collagen density of infarcted rats were significantly increased compared with sham-operated animals (Table 1). Although LV weight was not modified by treatment, LV collagen density was significantly reduced by ivabradine 3 and 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ . In addition, ivabradine significantly increased LV capillary density from  $85 \pm 6$  capillaries per field in untreated CHF rats to  $103 \pm 4$  capillaries per field in rats treated at 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ .

### Catecholamine Levels

After 90 days, noradrenaline plasma levels were increased by 122% in the untreated CHF group. Ivabradine significantly reduced noradrenaline levels by 15% and 16% at 3 and 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , respectively (Table 1).

### Effects of Interruption of Ivabradine Treatment

Three days after interruption of the 90-day ivabradine treatment at the dose of 10  $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ , heart rate rose significantly (13% compared with the value observed before interruption) and became similar to the value observed in the untreated CHF group. Compared with untreated CHF rats, LV end-systolic diameter remained reduced, whereas LV

TABLE 1. Cardiac Parameters and Noradrenaline Levels After 90 Days

	Sham	Untreated	CHF			
			Treatment With Ivabradine, mg · kg <sup>-1</sup> · d <sup>-1</sup>			
			0.3	1	3	10
LVSP, mm Hg	151±17	133±5	126±6	135±6	133±6	133±7
LVEDP, mm Hg	2.1±0.3*	16.8±3.6	14.8±3.3	15.8±3.3	10.2±3.0	11.5±4.0
LV dP/dt <sub>max</sub> , 10 <sup>3</sup> mm Hg/s	9.9±0.5*	7.0±0.4	7.0±0.4	7.0±0.5	7.3±0.4	7.6±0.4
LV dP/dt <sub>min</sub> , 10 <sup>3</sup> mm Hg/s	9.1±0.6*	5.0±0.4	4.7±0.4	4.6±0.5	5.6±0.4	5.8±0.8
LV weight, g	0.88±0.02*	1.05±0.04	1.13±0.05	1.07±0.03	1.14±0.05	1.14±0.03
LV collagen density, %	2.8±0.1*	3.4±0.1	3.3±0.1	3.4±0.1	3.1±0.1*	3.0±0.1*
Plasma noradrenaline, pmol/L	10.8±0.7*	24.0±1.0	23.8±1.3	22.8±0.9	20.4±1.1*	20.1±0.8*

LVSP indicates LV systolic pressure; LVEDP, LV end-diastolic pressure.

n=6 to 20 animals per group.

\*P<0.05 vs untreated CHF.

fractional shortening, posterior wall thickening, and stroke volume remained significantly increased after interruption of treatment. These effects resulted in a significant increase in cardiac output. Furthermore, systolic blood pressure, LV end-systolic and end-diastolic pressures, and LV dP/dt<sub>max</sub> and dP/dt<sub>min</sub> were not modified (Table 2). Moreover, the beneficial cardiac effects of ivabradine observed in vitro with the Langendorff preparation persisted after interruption of the treatment (Figure 5).

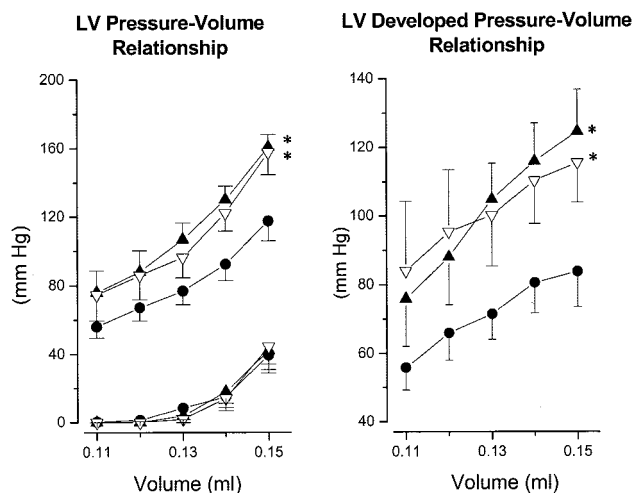
## Discussion

This study, using a rat model of CHF, shows that chronic administration of the selective I<sub>f</sub> current inhibitor ivabradine induces a dose-dependent reduction of heart rate without modifying systemic hemodynamics. Moreover, cardiac output is preserved despite the decrease in heart rate because stroke volume is increased owing to a decrease in LV end-systolic diameter without modification of LVEDD. This

improvement in LV function might be related in part to a modification of the LV structure and/or myocyte properties. This hypothesis is strengthened by the leftward shift of the LV systolic pressure–volume/developed pressure–volume relationships observed in vitro with the Langendorff preparation, the reduction of cardiac collagen accumulation in ivabradine-treated animals, and the persistence of the improvement in LV function despite normalization of heart rate after interruption of long-term treatment.

The effects of ivabradine have been studied in a context of severe heart failure. Indeed, the reduced blood pressure, major LV dilatation, and increased LV end-diastolic pressure, as well as diminished cardiac output, illustrate the deteriorated pathophysiological status of the untreated animals throughout the study, in agreement with previous studies.<sup>17–20</sup>

In this context of CHF, ivabradine induces a specific dose-dependent HRR. This pharmacological effect of this



**Figure 5.** LV pressure-volume and LV developed pressure-volume relationships as measured by LV systolic and LV end-diastolic pressures in response to increasing LV volume in untreated CHF rats (●), rats treated for 90 days with ivabradine at 10 mg · kg<sup>-1</sup> · d<sup>-1</sup> (▲), or 3 days after interruption of 90-day ivabradine administration (▽) (n=9 to 15 per group). \*P<0.05 vs untreated CHF.

TABLE 2. Cardiac Parameters Before and 3 Days After Interruption of 90-Day Treatment Period

	Untreated CHF	CHF Treated With Ivabradine (10 mg · kg <sup>-1</sup> · d <sup>-1</sup> )	
		Before Interruption	After Interruption
Heart rate, bpm	364±9	299±8*	338±13†
LVSP, mm Hg	133±5	133±7	135±9
LVEDP, mm Hg	16.8±3.6	11.5±4.0	20.0±3.8
LV dP/dt <sub>max</sub> , 10 <sup>3</sup> mm Hg/s	7.0±0.4	7.6±0.4	6.9±0.8
LV dP/dt <sub>min</sub> , 10 <sup>3</sup> mm Hg/s	5.0±0.4	5.8±0.9	4.2±0.7
LVEDD, mm	10.5±0.1	10.9±0.2	10.7±0.2
LV systolic diameter, mm	9.4±0.2	8.9±0.3	8.9±0.3
LV fractional shortening, %	10±1	19±1*	17±1*
LV posterior wall thickening, %	31±5	55±6*	62±8*
Stroke volume, mL/beat	0.41±0.03	0.49±0.03*	0.49±0.03*
Cardiac output, mL/min	163±11	140±9	177±10†

LVSP indicates LV systolic pressure; LVEDP, LV end-diastolic pressure; and LVEDD, LV end-diastolic diameter.

n=7 to 12 animals per group.

\*P<0.05 vs untreated CHF; †P<0.05 vs before interruption.



selective  $I_f$  inhibitor is independent of the pathophysiological status, because ivabradine induces a similar HRR in normal rats.<sup>9</sup>

It should be stressed that despite the marked HRR, ivabradine does not modify blood pressure. This, together with the fact that cardiac output is maintained, suggests that peripheral vascular resistance does not change. This absence of variation of peripheral vascular resistance is predictable, because ivabradine does not show vascular tropism in terms of vascular relaxation or contraction.<sup>10</sup>

Moreover, in the context of an already impaired contractility, the chronic HRR induced by ivabradine results in an enhancement of LV contractile force, illustrated in vivo by the decrease in LV systolic diameter and in vitro by the higher LV systolic pressure and developed pressure in the Langendorff experiments. Thus, long-term effects contrast with the acute effects of ivabradine or zatebradine, because the drug does not modify cardiac contractility after acute administration.<sup>9,21,22</sup>

Several mechanisms might be involved in the preservation of cardiac output and enhancement of LV stroke volume and function. First, by prolonging diastolic time, HRR improves LV filling and thus increases stroke volume by the Frank-Starling mechanism. Second, the results of the present study suggest that modifications of LV structure, which occur secondarily to long-term HRR, contribute to the improvement in cardiac function. Indeed, ivabradine reduces LV collagen accumulation after 90 days. Furthermore, LV end-systolic diameter but not LVEDD is reduced after long-term ivabradine administration. The hypothesis that long-term HRR might modify intrinsic myocardial structure is supported by the fact that 3 days after interruption of ivabradine administration after the 90-day treatment period, LV systolic diameter, function, and stroke volume each remained at the level observed before interruption, whereas heart rate rose to a level observed in the untreated heart failure group. Moreover, the improvement in LV function does not appear to be related to modifications in LV workload or to circulating neurohumoral factors, because the improvement in LV function was also observed in the isolated heart preparation at fixed preload and afterload conditions.

We can only speculate on the origins of the mechanisms that contribute to modification of myocardial structure and perhaps LV function. In this model, the "viable" part of the myocardium becomes ischemic and probably more prone to transient local hypoxia/ischemia/reperfusion injury, owing to a decrease in myocardial  $O_2$  supply related to capillary rarefaction<sup>23</sup> and an increase in  $O_2$  consumption mainly related to ventricular dilatation, an increase in LV end-diastolic pressure, and myocardial tension. The fact that long-term ivabradine administration induces myocardial angiogenesis, as described in normal animals for the bradycardic agent alinidine,<sup>24</sup> and thus prevents coronary rarefaction suggests that ivabradine probably augments myocardial perfusion. Moreover, because the heart rate–diastolic time relation is nonlinear, the decrease in heart rate induced by ivabradine most likely results in an increase in the diastolic part of the cardiac cycle,<sup>25</sup> leading to an enhanced coronary perfusion time and thus increasing myocardial perfusion, especially to

the deeper layer.<sup>26</sup> Finally, an increase in coronary perfusion due to HRR would prevent the development of coronary endothelial dysfunction provoked by the long-term decreased perfusion observed in CHF<sup>27,28</sup> and, by preventing local hypoxia, could diminish the local production of cytokines such as interleukin-1, interleukin-6, and tumor necrosis factor- $\alpha$ ,<sup>29</sup> free radicals,<sup>30</sup> and/or vasoconstrictors such as endothelin,<sup>31</sup> all factors implicated in the deterioration of LV function/remodeling and intrinsic tissue structure. Furthermore, in terms of myocardial  $O_2$  demand and tissue protective effects, the improvement in LV filling induced by the decrease in heart rate may diminish the level of sympathetic activity, as suggested by the decrease in noradrenaline plasma levels. This probably limits downregulation of cardiac  $\beta$ -receptors and the direct toxic effect of noradrenaline and thus mimics in part the effect of  $\beta$ -blockers. All these direct and indirect myocardial effects, together with a reduced  $O_2$  requirement induced by HRR,<sup>32</sup> will improve the  $O_2$  supply/demand ratio, which should be beneficial in terms of coronary reserve. However, whether the effects can be translated to improved exercise tolerance or improved survival, which depends in part on myocardial function, is unknown.

In conclusion, in a model of CHF, long-term HRR induced by the selective  $I_f$  inhibitor ivabradine improves LV function and increases stroke volume, which results in preserved cardiac output despite the HRR. This improvement in cardiac function is probably related not only to the HRR itself but also to modifications of LV structure and/or myocyte properties secondary to long-term HRR.

## Acknowledgments

This study was supported by a grant from the Institut de Recherches Internationales Servier, Courbevoie, France. The authors thank Dr J-P Morin for his constructive assistance concerning the Langendorff experiments and Elian Abdelhouab for technical assistance.

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