0022-3565/01/2993-1133–1139$3.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 299:1133–1139, 2001

Vol. 299, No. 3
4248/947460
Printed in U.S.A.

Effects of Heart Rate Reduction with Ivabradine on Exercise-Induced Myocardial Ischemia and Stunning

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Received June 19, 2001; accepted September 6, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT

We investigated the effects of the selective bradycardic agent ivabradine, an I_f channel inhibitor, on exercise-induced ischemia and resulting myocardial stunning. Seven dogs were chronically instrumented to measure left ventricular (LV) wall thickening (Wth), aortic pressure and coronary blood flow (CBFv) (Doppler). Circumflex coronary artery stenosis was set up to suppress the increase in CBFv during a 10 min treadmill exercise. During exercise under saline, LVWth in the ischemic zone was depressed (−70 ± 4%) and a prolonged myocardial stunning was subsequently observed. Infusion of ivabradine started before exercise significantly reduced heart rate (HR) at rest (−22 ± 7%), during exercise (−33 ± 4%) and throughout the recovery period (−21 ± 2%). By reducing HR during exercise, ivabradine simultaneously improved LVWth compared with saline (14 ± 1% versus 7 ± 1%, respectively) and subendocardial perfusion (microspheres). This anti-ischemic effect was subsequently responsible for a strong decrease in the intensity and severity of myocardial stunning. All these beneficial effects were abolished when HR reduction during exercise was suppressed by atrial pacing. Interestingly, when ivabradine infusion was started after exercise, LVWth was still significantly enhanced and myocardial stunning strongly attenuated. This direct effect of ivabradine on the stunned myocardium disappeared when HR reduction was suppressed by atrial pacing at rest. In conclusion, this study demonstrates that ivabradine exerts an anti-ischemic effect that is responsible for subsequent protection against myocardial stunning. Furthermore, administration of ivabradine after the ischemic insult still improves LVWth of the stunned myocardium.

By reducing myocardial oxygen demand and increasing diastolic perfusion time, heart rate reduction induced by β-blockers or nondihydropyridine calcium channel blockers affords a well known protection against myocardial ischemia (Kloner et al., 1985; Matsuzaki et al., 1985). To date, β-blockers are widely used in the treatment of patients with myocardial ischemia but their negative inotropic properties, which participate to the metabolic sparing effect, might be deleterious. Indeed, esmolol was detrimental for the recovery of myocardial stunning when administered after ischemia (Przyklenk and Kloner, 1989). β-Blockade has been demonstrated to also be responsible for a paradoxical coronary vasoconstriction in dogs and humans, both at rest and during exercise (Bortone et al., 1990; Berdeaux et al., 1991). In this setting, selective bradycardic agents have been developed for several years as an alternative approach to reduce heart rate without inducing such adverse side effects.

These bradycardic agents inhibit the hyperpolarization-activated I_f channel of the pacemaker cells in the cardiac sinoatrial node. By increasing the duration of spontaneous depolarization, they induce a selective heart rate reduction (Goethals et al., 1993). Among these agents, zatebradine has been the most extensively investigated and has been shown to produce anti-ischemic effects when administered before exercise-induced ischemia (Guth et al., 1987). However, its administration after the ischemic insult failed to protect against myocardial stunning in anesthetized pigs (Soei et al., 1994). Other compounds have been developed, and to date ivabradine has been demonstrated to be the most selective inhibitor for I_f channel inhibition and to be devoid of any effect on the repolarization period (Thollon et al., 1994; Bois et al., 1996; Thollon et al., 1997). Furthermore, ivabradine alters neither myocardial contractility nor coronary vasomotion at rest and during exercise in normal dogs (Simon et al., 1995).

In the present study, we investigated the effects of ivabradine on regional myocardial contractility during a brief period of high flow ischemia and the subsequent stunning. For this purpose, we used an original experimental model of ischemia developed by our group, which combines a treadmill exercise and a partial coronary artery stenosis in dogs (Par-

This project was supported by Grant 99002301 from the Fondation de France. Patrice Colin was recipient of support from the Société Française de Pharmacologie.

ABBREVIATIONS: LV, left ventricle; Wth, wall thickening; CBFv, coronary blood flow; HR, heart rate; RMBFs, regional myocardial blood flows.

ent de Curzon et al., 1998, 2000, 2001). The effects of ivabradine were investigated when its administration was started either before exercise-induced ischemia or during the recovery period, i.e., during myocardial stunning. To specifically investigate the contribution of ivabradine-induced heart rate reduction, the experiments were performed both at spontaneous heart rate and under atrial pacing.

Materials and Methods

Surgical Preparation. The animal instrumentation and the ensuing experiments were conducted in accordance with the Declaration of Helsinki and the recommendations of the French Ministry of Agriculture (approval A 94-043-12). Seven dogs (17–32 kg) were anesthetized with pentobarbitone sodium (30 mg/kg, i.v.), intubated, and mechanically ventilated. Left thoracotomy (5th intercostal space) was performed. Filled fluid Tygon catheters were implanted in the descending thoracic aorta and left atrium for measurement of blood pressure and fluorescent microspheres injections, respectively. A polymeric silicone flexible catheter was introduced into the pulmonary artery for drug administration. A solid state pressure transducer (P7A, Konigsberg Instruments, Pasadena, CA) was introduced in the left ventricle (LV) through the apex. A 10 MHz Doppler flow probe (Crystal Biotech, Hopkinton, MA) and a pneumatic occluder were implanted on the circumflex coronary artery. One pair of ultrasonic crystals (5 MHz) was placed within the distribution of the circumflex coronary artery (ischemic zone), and the other one was placed within the distribution of the left anterior descending coronary artery (nonischemic zone) for LV wall thickening measurement. Bipolar electrodes were fixed on the left atria to allow pacing. All catheters and wires were exteriorized between the scapulae, and the pneumothorax was evacuated. Cefazolin (1 g, i.v.) and gentamycin (40 mg, i.v.) were administered before and at the end of surgery. Postoperative pain was treated with pro-paracetamol (1 g, i.v.).

Hemodynamic Measurements. Aortic and left atrial pressures were measured with a P23ID strain gauge transducer (Statham Instruments, Oxnard, CA). Because it was measured by a hydraulic technique, aortic pressure could not be accurately recorded during exercise. LV pressure was measured using the Konigsberg gauge, cross calibrated against measurements of systolic aortic and left atrial pressures. Circumflex coronary artery flow velocity was measured with a Doppler flowmeter (System 6, Triton Technology Inc., San Diego, CA).

Measurements of Regional Contractility. Wall thicknesses were obtained by an ultrasonic transit-time dimension gauge (Triton Technology Inc., San Diego, CA). To determine wall thickening, end-diastolic wall thickness was measured at the initiation of the upstroke of LV pressure tracing, and the end-systolic wall thickness was measured 20 ms before negative LV dP/dt. Percent wall thickening was defined as end-systolic thickness minus end-diastolic thickness times 100 divided by end-diastolic thickness.

Measurements of Regional Myocardial Blood Flows. Regional myocardial blood flows (RMBFs) were measured using the fluorescent microspheres technique (Parent de Curzon et al., 2001). Microspheres (3×10^6, 15 ± 1 μm diameter) labeled with fluorescent dyes (blue, blue-green, yellow, orange, red, crimson, or far-red) suspended in 0.02% Tween 80 solution, were sonicated for 20 to 30 min and vortexed. Arterial blood reference samples were withdrawn at a rate of 7.5 ml/min for a total of 120 s, and microspheres were injected and flushed with saline over a 20-s period via the left atrial catheter. At termination of the study, the animal was given heparin (5000 IU, i.v.) and a lethal dose of sodium pentobarbitone. The heart was excised and a dual perfusion with Evans blue and saline was performed (Parent de Curzon et al., 2001). The left ventricle was cut into three to four slices and further divided into endocardium, mid-myocardium, and epicardium in the nonischemic and ischemic zones.

Each myocardial and reference blood sample was processed to extract the fluorescence. Blood flow for each myocardial sample was calculated as: $Q_m = Q_r \times Int_m/Int_r$, where Q_m is myocardial blood flow (ml/min), Q_r is the reference blood flow rate (ml/min), Int_m is the fluorescence intensity in the myocardial sample, and Int_r is the fluorescence intensity in the reference blood sample. Blood flow was then divided by the sample weight and expressed as milliliters per minute per gram of myocardium. Mean transmural flow was calculated as the combined flow of the three layers. The subendocardial/subepicardial flow ratio was also calculated. Data were recorded continuously on an ES 1000 recorder (Gould Instruments Inc., Cleveland, OH), digitized on a computer, and analyzed using the data acquisition software HEM 3.2 (Notocord Systems, Croissy-sur-Seine, France).

Experimental Protocol. Three weeks after surgery, dogs were installed on a treadmill, and baseline parameters were recorded ("Base 1") (Fig. 1). A second set of measurements ("Base 2") was initiated 20 min later. A partial stenosis of the left circumflex coronary artery was performed using the pneumatic occluder without altering LV posterior wall thickening at rest. A treadmill exercise (10 min duration, 14 km/h, 13% slope) was then started. The stenosis was maintained during exercise to keep mean coronary blood flow velocity at its corresponding baseline value. The occluder was deflated at the end of exercise. All parameters were continuously recorded at baseline, during exercise, 2 h after, and at selected intervals during the recovery period. RMBFs were measured between the 6th and the 8th min of exercise.

In the first part of the protocol ("sequence A"), saline or ivabradine was administered immediately after Base 1. Ivabradine [3-(3-{[((7*S*)-3, 4-dimethoxybicyclo[4,2,0]octa-1,3,5-trien-7-yl)methyl]methylamino}propyl)-1,3,4,5-tetrahydro-7,8-dimethoxy-2*H*-3-benzazepin-2-

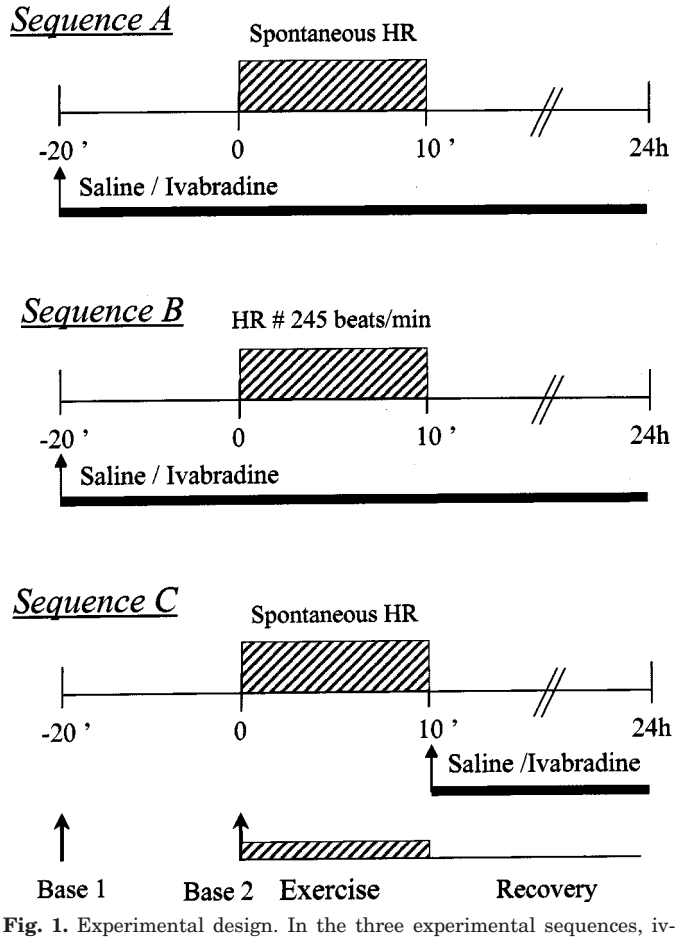

Fig. 1. Experimental design. In the three experimental sequences, ivabradine was administered intravenously at the dose of 1 mg/kg over 5 min followed by a continuous infusion of 0.5 mg/kg over 24 h.

one, hydrochloride; Laboratoires Servier, Neuilly-sur-Seine, France] was administered as an i.v. bolus (1 mg/kg over 5 min), followed by a continuous i.v. infusion (0.5 mg/kg/h) during 24 h using an automatic programmable pump (Zyklomat BT1, Ferring, Kiel, Germany) that was fixed on the back of the animal. The dose used for the i.v. bolus was chosen on the basis of a previous study, i.e., as that producing the highest reduction in exercise-induced tachycardia without inducing any major hemodynamic modification (Simon et al., 1995). The rate of the continuous i.v. infusion was calculated using the pharmacokinetic parameters determined in dogs. Nevertheless, we verified in preliminary experiments that heart rate reduction was constant over 24 h (data not shown). In the second part of the protocol ("sequence B"), the same design as in sequence A was used except that heart rate was kept constant during exercise by atrial pacing at 245 beats/min. In the third part of the protocol ("sequence C"), administration of either saline or ivabradine was started after exercise termination. Each recording made at rest before exercise and during the recovery period in the three sequences of the protocol was performed both at spontaneous heart rate and during a 1-min episode of atrial pacing at 150 beats/min to individualize the effects of heart rate reduction per se at rest.

In summary, sequences A and B were performed to look for potential anti-ischemic effects of ivabradine during exercise. These two sequences were also designed to potentially evaluate the consequences of the anti-ischemic effect on the subsequent myocardial stunning. Sequence C was set up to investigate the effects of heart rate reduction on the already stunned myocardium, independently from the potential anti-ischemic properties of ivabradine. An interval of at least 4 to 5 days was respected between each experiment in the same animal. All the experiments were performed in a random order.

Statistical Analysis. Data are reported as mean ± S.E.M. Comparisons of different parameters during exercises were performed using a two-way analysis of variance for repeated measures. Individual comparisons were analyzed using a paired Student's t test with a Bonferroni correction. A value of $p < 0.05$ was considered significant.

Results

These experiments were conducted in seven chronically instrumented dogs. Due to technical problems, only six, five, and six animals were included in sequences A, B, and C, respectively.

Heart Rate. As shown in Table 1, heart rate increased from 119 ± 8 to 220 ± 10 beats/min during the exercise performed under saline (sequence A). Ivabradine reduced heart rate by 18 ± 5% from 113 ± 5 beats/min before exercise and strongly attenuated the exercise-induced tachycardia as compared with saline (149 ± 12 versus 220 ± 10 beats/min, respectively). Throughout the 24-h recovery period, ivabradine infusion produced a constant heart rate reduction (−21 ± 2% versus saline).

In sequence B because of atrial pacing, heart rate during exercise was similar under saline and ivabradine. At rest (i.e., before exercise and during the recovery period), ivabradine reduced heart rate similarly as in sequence A (data not shown). In sequence C, administration of ivabradine during the recovery period reduced heart rate to a similar extent as in sequences A and B (data not shown).

Hemodynamics. As shown in Table 1, none of the hemodynamic parameters, i.e., mean arterial pressure, LV pressure, LV end-diastolic pressure, and maximum first derivative of left ventricular pressure, was affected by ivabradine at rest and during exercise compared with saline in sequence A.

TABLE 1
Effects of saline (control) and ivabradine on heart rate and hemodynamic parameters during sequence A
Parameters were measured at spontaneous heart rate. Mean ± S.E.M., $n = 6$.

	Base 1	Base 2	Exercise	Recovery					
				10 min	30 min	1 h	3 h	6 h	10 h	24 h
HR, beats/min										
Control	117 ± 8	119 ± 8	220 ± 10	148 ± 6	135 ± 4	131 ± 8	133 ± 9	124 ± 6	117 ± 8	118 ± 7
Ivabradine	113 ± 5	93 ± 8*	149 ± 12*	104 ± 7*	101 ± 7*	103 ± 8*	106 ± 8*	104 ± 11*	99 ± 8*	95 ± 5*
MAP, mm Hg										
Control	115 ± 7	114 ± 6		117 ± 7	111 ± 8	114 ± 7	120 ± 9	112 ± 8	110 ± 7	112 ± 8
Ivabradine	106 ± 11	109 ± 10		105 ± 9	107 ± 10	105 ± 12	111 ± 9	103 ± 9	98 ± 11	107 ± 17
LVP, mm Hg										
Control	142 ± 12	151 ± 13	164 ± 11	145 ± 12	142 ± 13	146 ± 14	147 ± 13	142 ± 12	146 ± 12	149 ± 13
Ivabradine	135 ± 16	140 ± 16	148 ± 17	138 ± 14	142 ± 14	144 ± 17	141 ± 14	132 ± 10	137 ± 15	147 ± 19
LVEDP, mm Hg										
Control	11 ± 1	12 ± 1	22 ± 3	12 ± 2	13 ± 2	10 ± 2	11 ± 2	11 ± 1	12 ± 2	10 ± 2
Ivabradine	9 ± 2	11 ± 2	19 ± 3	11 ± 2	11 ± 1	10 ± 1	11 ± 2	10 ± 1	11 ± 2	13 ± 2
dP/dt max, mm Hg/s										
Control	3706 ± 387	4212 ± 551	7296 ± 460	3845 ± 498	3629 ± 423	3940 ± 343	3433 ± 343	3573 ± 366	3774 ± 360	3716 ± 391
Ivabradine	3698 ± 551	3998 ± 739	6190 ± 805	4044 ± 628	4290 ± 714	4535 ± 906	3266 ± 511	3282 ± 352	3481 ± 444	4007 ± 501

MAP, mean arterial pressure; LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt max, maximum first derivative of left ventricular pressure.
* $p < 0.05$ significantly different from control.

Similar results were obtained in sequences B and C (data not shown).

LV Regional Myocardial Contractility. At rest, in sequence A, ivabradine tended to increase the nonischemic LV wall thickening and significantly increased the ischemic LV wall thickening before ischemia compared with saline when measured at spontaneous heart rate (31 ± 3% and 27 ± 4%, respectively). These effects were abolished under atrial pacing at 150 beats/min.

During exercise under saline in sequence A, LV wall thickening decreased dramatically in the ischemic zone compared with Base 1 value measured at spontaneous heart rate (−73 ± 4% from 27 ± 2%) or at 150 beats/min (−70 ± 4% from 24 ± 2%). This alteration in regional myocardial performance was significantly attenuated by ivabradine (LV wall thickening = 14 ± 1% versus 7 ± 1% under ivabradine and saline, respectively) (Table 2). In the nonischemic zone, LV wall thickening increased during exercise, and ivabradine significantly amplified this increase. In contrast, during sequence B (atrial pacing at 245 beats/min during exercise), LV wall thickening measured in the ischemic and nonischemic zones was similarly altered after ivabradine and saline administrations (3 ± 2% versus 3 ± 2%, 36 ± 7% versus 33 ± 5%, respectively).

After exercise in sequence A, although LV wall thickening returned rapidly to its corresponding baseline value in the nonischemic zone, it remained depressed in the ischemic zone under saline. Ivabradine significantly enhanced LV wall thickening measured at spontaneous heart rate in the ischemic zone during recovery, compared with saline (at 4 h LV wall thickening = 23 ± 2% versus 18 ± 1%, respectively). This effect was also observed if LV wall thickening was measured at 150 beats/min (Fig. 2). In sequence B, LV wall thickening measured at spontaneous heart rate was greater under ivabradine than under saline (at 4 h LV wall thickening = 29 ± 5% versus 21 ± 3%), but this effect disappeared if LV wall thickening was measured at 150 beats/min (Fig. 3). In sequence C, LV wall thickening measured at spontaneous heart rate was greater under ivabradine than under saline (Fig. 4), but as in sequence B, this effect was abolished by atrial pacing at 150 beats/min (Fig. 5).

LV Regional Myocardial Blood Flows. RMBFs measured at rest and during exercise in sequences A, B, and C are shown in Table 3. During exercise performed under saline (sequence A), transmural RMBFs in the nonischemic zone increased markedly. In contrast, transmural RMBFs remained unchanged in the ischemic zone due to coronary stenosis.

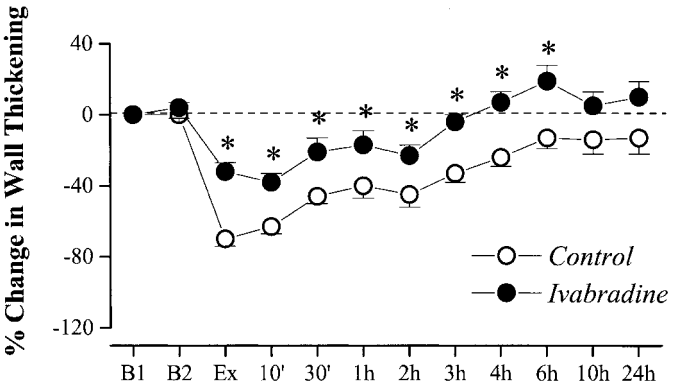

Fig. 2. Evolution of wall thickening (% change from Base 1) in the ischemic zone as measured before (B1) and after (B2) administration of saline (control) or ivabradine in sequence A. Recordings at baseline and during the recovery period were made under atrial pacing at 150 beats/min. *, $p < 0.05$: significantly different from the corresponding control value, $n = 6$.

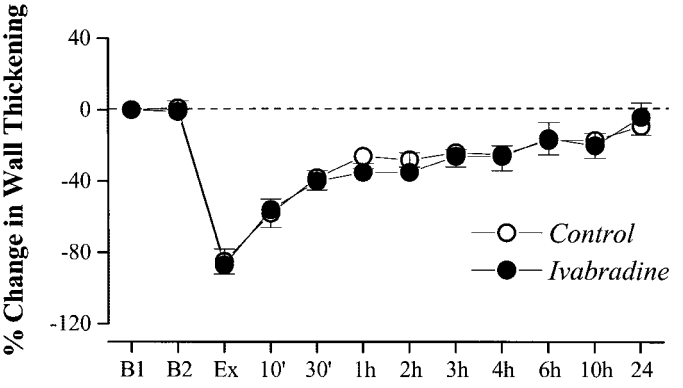

Fig. 3. Evolution of wall thickening (% change from Base 1) in the ischemic zone as measured before (B1) and after (B2) administration of saline (control) or ivabradine in sequence B when treadmill exercise (Ex) was performed under atrial pacing at 245 beats/min. Recordings at baseline and during the recovery period were made under atrial pacing at 150 beats/min. $p < 0.05$, significantly different from the corresponding control value, $n = 5$.

TABLE 2

Effects of saline (control) and ivabradine on regional contractility in the ischemic and nonischemic zones of the left ventricle during sequence A

Parameters were measured during atrial pacing at 150 beats/min. Mean ± S.E.M., $n = 6$.

	Base 1	Base 2	Exercise	Recovery						
				10 min	30 min	1 h	3 h	6 h	10 h	24 h
NIZ ED, mm										
Control	10.4 ± 1.1	10.3 ± 1.1	9.9 ± 1.2	10.5 ± 1.1	10.4 ± 1.1	10.3 ± 1.0	10.2 ± 1.2	10.3 ± 1.2	10.4 ± 1.1	11.0 ± 1.1
Ivabradine	10.8 ± 1.2	10.9 ± 1.4	9.7 ± 1.3	10.6 ± 1.2	10.7 ± 1.2	10.8 ± 1.3	10.9 ± 1.4	11.2 ± 1.4	11.1 ± 1.3	11.6 ± 1.2
NIZ Wth, %										
Control	20 ± 2	21 ± 2	36 ± 5	20 ± 3	22 ± 3	23 ± 3	21 ± 3	23 ± 3	23 ± 4	20 ± 3
Ivabradine	20 ± 3	18 ± 4	43 ± 5	25 ± 4	24 ± 4	24 ± 5	23 ± 5	21 ± 5	21 ± 3	19 ± 2
IZ ED, mm										
Control	10.2 ± 0.4	10.3 ± 0.4	9.7 ± 0.4	10.1 ± 0.4	10.3 ± 0.3	10.3 ± 0.4	10.1 ± 0.4	10.2 ± 0.5	10.5 ± 0.6	10.5 ± 0.5
Ivabradine	10.8 ± 0.5	10.6 ± 0.5	9.8 ± 0.5	10.4 ± 0.4*	10.6 ± 0.5	10.5 ± 0.4	10.0 ± 0.4	10.2 ± 0.4	10.3 ± 0.4	10.3 ± 0.4
IZ Wth, %										
Control	24 ± 2	24 ± 2	7 ± 1	9 ± 1	13 ± 1	14 ± 1	16 ± 1	21 ± 3	21 ± 3	20 ± 1
Ivabradine	21 ± 2	22 ± 2	14 ± 1*	13 ± 1*	17 ± 2*	18 ± 2*	20 ± 1*	25 ± 2*	22 ± 2	23 ± 2

NIZ ED, nonischemic zone end-diastolic wall thickness; NIZ Wth, nonischemic zone wall thickening; IZ ED, ischemic zone end-diastolic wall thickness; IZ Wth, ischemic zone wall thickening.

* $p < 0.05$ significantly different from control.

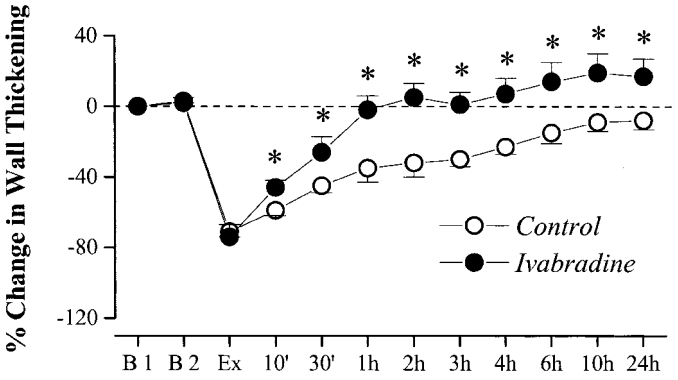

Fig. 4. Evolution of wall thickening (% change from Base 1) in the ischemic zone as measured before exercise (B1 and B2), during exercise (Ex), and during the recovery period in sequence C when saline (control) and ivabradine administrations were started at the end of the treadmill exercise. All measurements were performed at spontaneous heart rate. *, $p < 0.05$, significantly different from the corresponding control value, $n = 6$.

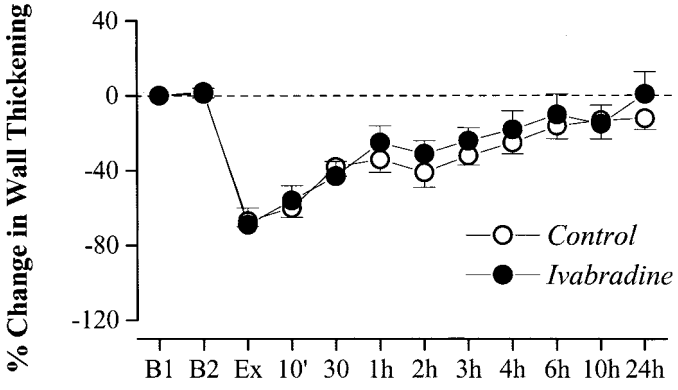

Fig. 5. Evolution of wall thickening (% change from Base 1) in the ischemic zone as measured before exercise (B1 and B2), during exercise (Ex), and during the recovery period in sequence C when saline (control) and ivabradine administrations were started at the end of the treadmill exercise. Recordings at baseline and during the recovery period were made under atrial pacing at 150 beats/min. $p < 0.05$, significantly different from the corresponding control value, $n = 6$.

Regardless of the LV zone, a classical transmural gradient of myocardial perfusion that favored the subendocardium was observed at baseline in the three sequences of experiments. During exercise performed under saline, this perfusion gradient decreased significantly in the ischemic zone whereas it remained unchanged in the nonischemic zone. RMBFs within the endocardium as well as the corresponding endocardium/epicardium ratio significantly increased during ivabradine administration compared with saline in sequence A. In sequence B, endocardial RMBF values and corresponding endocardium/epicardium ratios measured under ivabradine and saline were comparable. During sequence C, these parameters were not affected by ivabradine since the drug was administered after the end of exercise.

Discussion

This study demonstrates that the selective I_f channel inhibitor ivabradine enhances LV wall thickening in exercise-induced myocardial stunning. On the one hand, limitation of exercise-induced tachycardia is responsible for an anti-ischemic effect that attenuates the subsequent myocardial stunning. On the other hand, heart rate reduction per se enhances regional LV wall thickening of the stunned myocardium, as observed when the drug is administered after the ischemic insult.

As previously described (Homans et al., 1986; Parent de Curzon et al., 1998, 2000, 2001), the combination of treadmill exercise and coronary artery stenosis was responsible for the development of a severe regional imbalance between myocardial metabolic demand and oxygen supply, resulting in myocardial ischemia. This resulted in a strong decrease in regional contractility in the ischemic zone. At exercise completion, the stenosis was relieved, and all hemodynamic parameters returned to their baseline values within 30 min. Although we did not measure RMBFs during the recovery period, we previously demonstrated that myocardial perfusion rapidly returns to its baseline value after the end of exercise (Parent de Curzon et al., 1998, 2000, 2001). However, regional myocardial function in the ischemic zone remained longlastingly depressed, indicating myocardial stunning.

Administration of ivabradine before the onset of exercise significantly decreased heart rate at rest and dramatically limited the exercise-induced tachycardia. Heart rate reduction induced by ivabradine clearly improved subendocardial perfusion and myocardial regional function during ischemia, demonstrating an anti-ischemic effect of ivabradine in agreement with previous studies using zatebradine (Guth et al., 1987) or other selective bradycardic agents (Dämmgen et al., 1985; Gross and Dämmgen, 1986 and 1987; Indolfi et al., 1989). These effects were solely due to heart rate reduction since they were abolished by atrial pacing during exercise. An increase in the diastolic interval could also participate to the cardioprotective effect (Gout et al., 1992). Therefore, heart rate reduction induced by ivabradine leads to both an increase in myocardial oxygen supply and a decrease in metabolic demand.

To our knowledge, only one study previously investigated the effects of a selective bradycardic agent, zatebradine, on myocardial stunning (Raberger et al., 1987). In agreement with this study, administration of ivabradine was responsible for a significant decrease in the severity and intensity of the subsequent myocardial stunning. Since administration of ivabradine before ischemia at rest was responsible for an increase in LV wall thickening, we thought that this effect of heart reduction could represent a confounding factor for the interpretation of the subsequent stunning observed under saline and ivabradine. Accordingly, only recovery values measured under atrial pacing at 150 beats/min in sequences A and B were analyzed to draw such a conclusion. Therefore, since the beneficial effects on stunning of ivabradine were still observed at 150 beats/min in sequence A (Fig. 2) but not in sequence B (Fig. 3), we can conclude that the effects of ivabradine on myocardial stunning result solely from the anti-ischemic properties of the drug and cannot be attributed to other intrinsic effects on regional contractility of the stunned myocardium.

Administration of ivabradine after exercise was performed to assess the effects of the drug on regional contractility of the stunned myocardium, independent of its anti-ischemic effect. When all comparisons were performed at similar heart rates, i.e., 150 beats/min, no beneficial effects of ivabradine were observed when its administration was started after ischemia. Surprisingly, when the analysis was performed

TABLE 3
Regional myocardial blood flows (ml/min/g) in the ischemic and nonischemic zones of the myocardium measured at baseline and during exercise
Mean ± S.E.M., $n = 4$.

	Sequence A			Sequence B			Sequence C		
	Baseline	Ex under Saline	Ex under Ivabradine	Baseline	Ex under Saline	Ex under Ivabradine	Baseline	Ex before Saline	Ex before Ivabradine
Nonischemic zone									
Endo	1.12 ± 0.22	2.78 ± 0.26	2.02 ± 0.15	0.96 ± 0.30	2.05 ± 0.27	2.09 ± 0.51	1.02 ± 0.25	2.70 ± 0.31	2.45 ± 0.24
Mid	1.02 ± 0.22	2.28 ± 0.28	1.71 ± 0.19	0.90 ± 0.25	2.11 ± 0.20	1.95 ± 0.46	0.89 ± 0.26	2.34 ± 0.24	2.34 ± 0.46
Epi	0.74 ± 0.08	2.14 ± 0.33	1.51 ± 0.19	0.61 ± 0.18	2.13 ± 0.29	1.99 ± 0.34	0.70 ± 0.10	2.38 ± 0.21	1.73 ± 0.39
Transmural	0.96 ± 0.17	2.40 ± 0.28	1.71 ± 0.15	0.83 ± 0.23	2.09 ± 0.10	2.01 ± 0.41	0.87 ± 0.20	2.47 ± 0.23	2.17 ± 0.33
Endo/Epi	1.48 ± 0.14	1.34 ± 0.13	1.39 ± 0.18	1.74 ± 0.33	1.02 ± 0.21	1.06 ± 0.22	1.42 ± 0.15	1.14 ± 0.10	1.58 ± 0.39
Ischemic zone									
Endo	1.06 ± 0.11	0.78 ± 0.09	0.87 ± 0.09*	1.01 ± 0.14	0.67 ± 0.23	0.78 ± 0.14	1.04 ± 0.12	0.91 ± 0.15	0.87 ± 0.11
Mid	1.12 ± 0.23	1.09 ± 0.15	1.17 ± 0.10	0.87 ± 0.29	0.71 ± 0.14	0.86 ± 0.13	1.06 ± 0.25	1.23 ± 0.24	1.11 ± 0.14
Epi	0.94 ± 0.21	1.28 ± 0.15	1.21 ± 0.13	0.94 ± 0.32	0.97 ± 0.25	1.29 ± 0.13	0.90 ± 0.22	1.41 ± 0.26	1.41 ± 0.13
Transmural	1.04 ± 0.18	1.05 ± 0.12	1.08 ± 0.05	0.94 ± 0.24	0.81 ± 0.17	0.97 ± 0.07	1.00 ± 0.19	1.18 ± 0.22	1.13 ± 0.09
Endo/Epi	1.18 ± 0.12	0.62 ± 0.06	0.73 ± 0.08*	1.21 ± 0.18	0.66 ± 0.11	0.63 ± 0.15	1.21 ± 0.12	0.65 ± 0.02	0.63 ± 0.10

Ex, exercise; Endo, endocardium; Epi, epicardium; Mid, mid-myocardium.
* $p < 0.05$ significantly different from saline.

with parameters measured at spontaneous heart rate, we observed a strong enhancement of LV wall thickening in the previously ischemic zone. To our knowledge, this is the first time that heart rate reduction is demonstrated to improve regional contractility of the stunned myocardium, i.e., when ivabradine is administered after the ischemic insult. Although we did not specifically investigate the mechanism(s) involved, this finding is probably the consequence of a Frank-Starling mechanism secondary to changes in loading conditions induced by heart rate reduction. Indeed, LV wall thickening was increased at baseline by ivabradine, in agreement with Raberger et al. (1987). Furthermore, Guth et al. (1987) also reported that administration of zatebradine could improve myocardial contractility during exercise by a Frank-Starling mechanism. Interestingly, Fan et al. (1995) and Schulz et al. (2000) demonstrated that the stunned myocardium is highly sensitive to loading conditions. In contrast, Soei et al. (1994) reported that zatebradine was unable to improve segment shortening when administered during myocardial stunning induced by an acute coronary artery occlusion followed by reperfusion in pigs. Differences in experimental design and in animal species, such as those previously described for stunning (Shen and Vatner, 1996), may explain this discrepancy.

Finally, it should be considered that repetition of exercise-induced ischemia might have induced a late preconditioning phenomenon (Sun et al., 1995), which could have influenced our findings. However, this is unlikely because all experiments were performed every 4 to 5 days in each animal, and it is reasonable to consider that after this period of time, the endogenous protecting mechanisms have almost vanished (Tang et al., 1996). We previously demonstrated that, in contrast with experimental models of coronary artery occlusion followed by reperfusion, exercise-induced ischemia does not induce any late preconditioning against myocardial stunning (Parent de Curzon et al., 2001). Therefore, we believe that our results cannot be accounted for by late preconditioning against myocardial stunning.

In conclusion, this study demonstrates that the specific I_f channel inhibitor ivabradine exerts an anti-ischemic effect also responsible for subsequent protection against myocardial stunning. Interestingly, administration of ivabradine after the ischemic insult improves the regional contractility of the stunned myocardium. All these beneficial effects are due to heart rate reduction, i.e., an improvement in the oxygen supply/demand balance when used as an anti-ischemic agent, and probably to a Frank-Starling mechanism when administered after ischemia. These results extend to myocardial stunning, the fundamental importance of controlling heart rate previously demonstrated in heart failure (Lechat et al., 1998), and myocardial infarction (Hjalmarson et al., 1990).

Acknowledgments

We thank Drs. Florence Mahlberg-Gaudin and Guy Lerebourg from Laboratoires Servier for fruitful discussion during the elaboration of this manuscript. Alain Bizé provided excellent technical assistance, and Stéphane Bloquet provided animal care.

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