European Heart Journal (2004) **25**, 579–586

ELSEVIER

EUROPEAN SOCIETY OF CARDIOLOGY

Preclinical research

Heart rate reduction during exercise-induced myocardial ischaemia and stunning

Xavier Monnet[1,a], Patrice Colin[1,a], Bijan Ghaleh[a], Luc Hittinger[b], Jean-François Giudicelli[a], Alain Berdeaux[a,*]

[a] *Laboratoire de Pharmacologie, INSERM E 00.01, Faculté de Médecine Paris Sud, Paris, France*
[b] *Fédération de Cardiologie, Hôpital Henri Mondor, Créteil, France*

Received 20 March 2003; received 18 September 2003; accepted 16 October 2003

See page 537 for the editorial comment on this article[†]

KEYWORDS
Chronotropic agent;
Heart rate;
Ischaemia;
Myocardial stunning;
Ventricular function

Aims The respective contributions of reduction in heart rate and inotropism in the beneficial effects of beta-blockade in ischaemic heart disease remains debated. The effects of selective heart rate reduction by ivabradine ($I_f$inhibitor) were compared to those of atenolol on exercise-induced ischaemia and stunning.
Methods and results In seven instrumented dogs, coronary stenosis was calibrated to suppress increase in coronary blood flow during a 10-min treadmill exercise. When administered before exercise, atenolol and ivabradine similarly reduced heart rate versus saline at rest and during exercise (154 ± 2 and 155 ± 9 vs 217 ± 13 beats/min, respectively). During exercise, left ventricular wall thickening (LVWth) was reduced to $2 \pm 1\%$ from $23 \pm 4\%$ under saline but ivabradine limited this effect ($10 \pm 3\%$) and reduced the subsequent myocardial stunning vs saline. Atenolol also limited LVWth decrease during exercise ($17 \pm 4\%$) but had no effect during recovery. When administered after exercise, ivabradine attenuated stunning and this effect disappeared when heart rate reduction was corrected by atrial pacing. Atenolol administered after exercise severely depressed LVWth vs saline.
Conclusion Selective heart rate reduction not only provides an anti-ischaemic effect but also per se improves contractility of the stunned myocardium. Additional negative inotropism is protective against ischaemia but deleterious during stunning.

Introduction

Heart rate is a major and independent predictor of cardiovascular morbidity and mortality in ischaemic heart disease.[1] Among drugs which reduce heart rate, β-blockers also decrease myocardial contractility and oxygen consumption, and improve diastolic perfusion time.[2,3] However their negative inotropic effect might be deleterious when left ventricular dysfunction occurs, e.g. during myocardial stunning,[4] although conflicting results have been reported on this issue.[5] Furthermore, the anti-ischaemic benefit of negative inotropism is not well established.[6,7] In this setting, agents which selectively reduce heart rate have been developed as an alternative approach. They provide a potent anti-ischaemic effect and strongly protect the myocardium against stunning in ischaemic exercising dogs.[8,9]

Accordingly, the aim of this study was to compare on the ischaemic and stunned myocardium the effects of

[1] X. Monnet and P. Colin both contributed equally to this work.
* Correspondence to: Pr. Alain Berdeaux, Laboratoire de Pharmacologie, INSERM E 00.01, Faculté de Médecine Paris-Sud, 63, rue Gabriel Péri, 94270 Le Kremlin-Bicêtre, France. Tel.: +33-1-495967021; fax: +33-1-49600031.
E-mail address: alain.berdeaux@kb.u-psud.fr (A. Berdeaux).
† doi:10.1016/j.ehj.2003.11.005.

0195-668X/$ - see front matter
doi:10.1016/j.ehj.2003.10.010

the widely used β-blocker, atenolol, to those of ivabradine, a novel selective inhibitor of the cardiac pacemaker hyperpolarization-activated I_f channel in the cardiac sino-atrial node.[10–12] By increasing the duration of spontaneous depolarization, it induces a selective heart rate reduction[10] and its administration is devoid of any effect on myocardial contractility and coronary vasomotion.[13] For this purpose, we used an experimental model in which ischaemia resulted from the combination of a treadmill exercise and a partial coronary artery stenosis in conscious dogs.[9,14] The effects of ivabradine and atenolol were investigated when their administration was started either before exercise-induced ischaemia or during the recovery period, i.e., during myocardial stunning. To specifically investigate the contribution of heart rate reduction, the experiments were performed both at spontaneous heart rate and under atrial pacing.

Methods

The animal instrumentation and the ensuing experiments were conducted in accordance with the recommendations of the French Ministry of Agriculture.

Surgical preparation

As previously described,[9] a left thoracotomy was performed in seven dogs (22—29 kg). Filled fluid catheters were implanted in the descending thoracic aorta and the left atrium for measurement of blood pressure and microspheres injections, respectively. A Silastic catheter was introduced into the pulmonary artery for drug administration. A solid-state micromanometer (Konigsberg Instruments, Pasadena CA, USA) was introduced into the left ventricle (LV). A Transonic flow probe and a pneumatic occluder were implanted on the circumflex coronary artery. Two pairs of ultrasonic crystals were placed within the distribution of the circumflex coronary artery (ischaemic zone) and of the left anterior descending coronary artery (non-ischaemic zone) for LV wall thickening measurement. Electrodes were fixed on the left atrial appendage for pacing. All catheters and wires were exteriorized between the scapulae. Cefazolin (1 g, i.v.) and gentamycin (40 mg, i.v.) were administered before and during the first week after surgery. Post-operative analgesia was provided with morphine.

Haemodynamic measurements

Data were recorded and analysed using the data acquisition software Notocord-HEM 3.3 (Notocord System, Croissy-sur-Seine, France). Aortic and left atrial pressures were measured with a Statham P23ID strain gauge transducer (Gould-Nicolet, Courtaboeuf, France). 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 and LV dP/dt was computed from the LV pressure signal. Circumflex coronary artery blood flow was measured with a transit-time flowmeter (Transonic T206, Transonic Systems, Ithaca, NY, USA). Diastolic perfusion time was measured from negative LV dP/dt to theinitiation of the upstroke of LV pressure tracing.

Measurements of regional contractility

According to the method used in our laboratory,[15] wall thicknesses were obtained using sonomicrometry, i.e., with an ultrasonic transit-time dimension gauge. The sonomicrometer (Module 201, System 6, Triton Technology Inc., San Diego, CA, USA) measures the distance between the endocardial and epicardial crystals by measuring the transit time of ultrasound between this pair of crystals and converting this time to an equivalent distance, the ultrasonic impulses travelling at the velocity of 1.58 μm/s. 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 minus end-diastolic thicknesses times 100 and divided by end-diastolic thickness.

Measurements of regional myocardial blood flows

As previously reported,[14] regional myocardial blood flows (RMBFs) were measured using the fluorescent microspheres technique. Microspheres labelled with fluorescent dyes (FluoSpheres, Triton System, San Diego, CA, USA) were injected via the left atrial catheter. Arterial blood reference samples were withdrawn (7.5 ml/min during 120 s). At termination of the study, the heart was excised and the left ventricle was cut into 3—4 slices and further divided into endocardium, mid-myocardium and epicardium in the non-ischaemic and ischaemic zones. Samples were then processed and blood flows (expressed as ml/min/g of myocardium) were calculated.

Experimental protocol

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

In the first part of the protocol ('sequence A'), saline, atenolol or ivabradine (Laboratoires Servier, Neuilly-sur-Seine, France) were administered immediately after Base 1. This sequence was performed to evaluate the potential anti-ischaemic effects of atenolol and ivabradine during exercise and their consequences on subsequent myocardial stunning during the recovery period. Ivabradine 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 6 h using an automatic programmable pump which was fixed on the back of the animal. We previously demonstrated that this regimen of administration of the drug induces a significant heart rate reduction which remains stable during the infusion period.[9] Atenolol was administered as an i.v. bolus (1 mg/kg over 5 min). As previously reported,[16] the regimen of ivabradine and atenolol provide similar heart rate reduction at rest and during exercise. In the second part of the protocol ('sequence B'), administration of atenolol or ivabradine was started immediately after the end of exercise. Sequence B was set up to investigate the direct effects of heart rate

reduction on the already stunned myocardium, thus independently from the potential anti-ischaemic properties of the drugs.

Each recording made at rest before exercise and during the recovery period was performed both at spontaneous heart rate and during a 1-min episode of atrial pacing at 150 beats/min in order to individualize the effects of heart rate reduction per se. Each animal performed the five experimental sessions (sequence A with saline, ivabradine, atenolol and sequence B with ivabradine and atenolol) in random order with at least a 5-day interval.

Statistical analysis

Data are reported as mean ± SEM. The experiments were conducted as an incomplete design in which each dog received all five treatments in a randomized order. Data during stunning were analysed using two-way ANOVA for repeated measures (repeated times nested in treatments) and by checking for interactions. One way ANOVA were performed to analyse baseline values. The Fisher–Snedecor test was used to test the significance of analysis of variance. When needed, pairwise comparisons between (a) saline and atenolol, (b) saline and ivabradine and (c) ivabradine and atenolol were performed using a paired Student t-test with the Bonferroni correction. Analyses were performed separately for experimental designs under sequence A and those under sequence B. Significance was accepted at $P < 0.05$.

Results

Heart rate

Values of heart rate at Base 1 were not significantly different and no further interaction was detected using ANOVA.

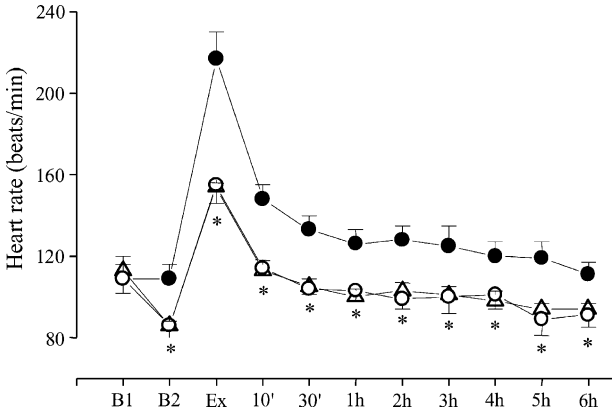

Fig. 1 Evolution of heart rate measured before (B1) and after administration (B2) of saline (full circles), ivabradine (open circles) or atenolol (open triangles) in sequence A. *$P < 0.05$: atenolol and ivabradine significantly different from saline.

As illustrated in Fig. 1, heart rate increased from 109 ± 7 to 217 ± 13 beats/min during exercise performed under saline in sequence A. Heart rate was similarly reduced by atenolol and ivabradine at rest (86 ± 2 and 86 ± 7 beats/min, respectively) and during exercise (154 ± 2 and 155 ± 9 beats/min, respectively). Throughout the recovery period, heart rate under atenolol and ivabradine remained constant and significantly reduced ascompared to saline (e.g. at 1 h, 100 ± 4 and 103 ± 5 vs 126 ± 7 beats/min, respectively). As shown in Table 1, heart rate values were similar between saline, atenolol and ivabradine during the 1 min of atrial pacing performed for each data recording at rest and during recovery.

In sequence B, as drugs were administered during the recovery period, heart rate values were similar at rest

Table 1 Effects of saline, atenolol and ivabradine on heart rate and haemodynamic parameters during sequence A. Parameters were measured at 150 beats/min (except during exercise)

	Recovery									
	Base 1	Base 2	Exercise	10 min	30 min	1 h	2 h	3 h	4 h	6 h
HR, beats/min										
saline	151 ± 1	152 ± 0	217 ± 13	154 ± 3	150 ± 2	153 ± 1	150 ± 1	153 ± 1	153 ± 2	154 ± 3
atenolol	151 ± 1	150 ± 1	154 ± 2[a]	151 ± 1	151 ± 1	151 ± 1	151 ± 1	151 ± 1	151 ± 1	150 ± 1
ivabradine	151 ± 1	150 ± 1	155 ± 9[a]	154 ± 3	149 ± 2	146 ± 6	153 ± 1	151 ± 1	150 ± 1	150 ± 2
MAP, mmHg										
saline	94 ± 4	94 ± 6	–	88 ± 7	91 ± 7	90 ± 6	97 ± 8	96 ± 7	98 ± 8	89 ± 7
atenolol	85 ± 8	76 ± 10[a,b]	–	72 ± 10[a,b]	78 ± 9[a,b]	74 ± 9[a,b]	81 ± 8[a,b]	82 ± 8[a,b]	90 ± 9[a,b]	77 ± 10[a,b]
ivabradine	92 ± 7	97 ± 6	–	95 ± 7	91 ± 6	91 ± 6	97 ± 6	99 ± 6	98 ± 7	95 ± 5
LVP, mmHg										
saline	123 ± 5	124 ± 4	150 ± 8	120 ± 6	118 ± 3	123 ± 5	126 ± 7	123 ± 6	126 ± 7	120 ± 6
atenolol	125 ± 8	126 ± 7	137 ± 10	122 ± 8	123 ± 8	125 ± 8	127 ± 7	121 ± 7	117 ± 7	118 ± 6
ivabradine	126 ± 4	134 ± 4	160 ± 7	128 ± 6	123 ± 5	122 ± 6	123 ± 6	126 ± 6	124 ± 6	127 ± 6
LVEDP, mmHg										
saline	3 ± 1	6 ± 2	24 ± 3	7 ± 2	7 ± 1	4 ± 1	7 ± 2	4 ± 1	5 ± 1	3 ± 1
atenolol	5 ± 2	7 ± 3	27 ± 3	4 ± 2	6 ± 2	6 ± 2	5 ± 1	3 ± 1	4 ± 2	4 ± 2
ivabradine	5 ± 2	6 ± 3	33 ± 5	7 ± 3	3 ± 2	4 ± 1	4 ± 1	5 ± 1	4 ± 1	6 ± 1

Mean ± SEM., $n = 7$, HR: heart rate; MAP: mean arterial pressure; LVP: left ventricular pressure; LVEDP: left ventricular end diastolic pressure.
[a] $P < 0.05$ significantly different from saline.
[b] $P < 0.05$ atenolol significantly different from ivabradine.

and during exercise among the saline, atenolol and ivabradine experiments. During recovery, the reduction in heart rate was similar under atenolol and ivabradine as compared to sequence A (e.g. at 1 h, 106 ± 6 and 101 ± 8 vs 126 ± 7 beats/min, respectively).

Haemodynamics

Values of mean arterial pressure, left ventricular pressure and end-diastolic left ventricular pressure measured at Base 1 were not significantly different and no further interaction was detected using ANOVA.

As heart rate reduction can per se modify LV wall thickening,[9] it represents a confounding factor in the interpretation of the results and accordingly in sequence A, only values measured under atrial pacing (150 beats/min) at baseline and during the recovery period were analysed.

In sequence A during atrial pacing (Table 1), mean arterial pressure was significantly reduced at rest, during exercise and the recovery period with administration of atenolol but not ivabradine. LV end-diastolic pressure and LV pressure were not significantly different among saline, atenolol and ivabradine.

In sequence B, administration of atenolol and ivabradine at exercise completion produced similar effects to those described in sequence A.

LV wall thickening in the ischaemic zone

Values of LV wall thickening and end-diastolic wall thickness measured at Base 1 were not significantly different. However, significant interactions treatment x time were detected using ANOVA for LV wall thickening during the stunning period in both sequences A and B.

In sequence A, at Base 2 during atrial pacing under atenolol but not ivabradine, LV wall thickening was significantly reduced as compared to saline (Table 2, Fig. 2). These effects were similar at spontaneous heart rate except that LV wall thickening was significantly increased in the session with ivabradine (data not shown). During exercise under saline, LV wall thickening decreased dramatically to $2 \pm 1\%$ from $23 \pm 4\%$ but this decrease was significantly reduced in the session with ivabradine (LV wall thickening: $10 \pm 3\%$) and to a significantly greater extent in the session with atenolol (LV wall thickening: $17 \pm 4\%$). During the recovery period, LV wall thickening measured during atrial pacing remained depressed for several hours under saline, indicating myocardial stunning. Values under atenolol were similar to those measured under saline. In contrast, LV wall thickening was significantly improved under ivabradine throughout the recovery period. Similar effects were observed at spontaneous heart rate (data not shown).

In sequence B, LV wall thickening was similar at Base 2 and during exercise before the administration of the drugs. During recovery, LV wall thickening was significantly greater under ivabradine than saline anddepressed in the session with atenolol at spontaneous heart rate (Fig. 3). Under atrial pacing, LV wall thickening was similar between saline and ivabradine but still significantly reduced after administration of atenolol (Fig. 4).

LV wall thickening in the non-ischaemic zone

Values of LV wall thickening and end-diastolic wall thickness measured at Base 1 were not significantly dif-

Table 2 Effects of saline, atenolol and ivabradine on regional contractility in the ischaemic and non ischaemic zones of the left ventricle during sequence A. Parameters were measured at 150 beats/min (except during exercise)

	Recovery									
	Base 1	Base 2	Exercise	10 min	30 min	1 h	2 h	3 h	4 h	6 h
NIZ ED, mm										
saline	9.0 ± 0.8	9.2 ± 0.8	8.0 ± 0.9	8.6 ± 0.8	8.8 ± 0.8	8.9 ± 0.8	8.9 ± 0.9	8.8 ± 0.8	8.8 ± 0.8	8.9 ± 0.7
atenolol	8.6 ± 0.8	$8.4 \pm 0.9^{a,b}$	7.5 ± 0.9	8.2 ± 0.8	8.2 ± 0.8	8.4 ± 0.8	8.3 ± 0.9	8.2 ± 0.8	8.4 ± 0.8	8.6 ± 0.9
ivabradine	8.9 ± 0.8	8.8 ± 0.8^{a}	7.8 ± 0.9	8.7 ± 0.9	8.8 ± 0.9	8.9 ± 0.9	8.6 ± 0.9	8.7 ± 0.9	8.7 ± 0.9	8.6 ± 0.9
NIZ Wth, %										
saline	32 ± 3	28 ± 2	52 ± 9	35 ± 4	34 ± 6	33 ± 4	32 ± 5	30 ± 4	28 ± 3	30 ± 4
atenolol	30 ± 3	$24 \pm 2^{a,b}$	$34 \pm 6^{a,b}$	$28 \pm 3^{a,b}$	$26 \pm 3^{a,b}$	$25 \pm 3^{a,b}$	$23 \pm 3^{a,b}$	$24 \pm 2^{a,b}$	$23 \pm 2^{a,b}$	$25 \pm 2^{a,b}$
ivabradine	31 ± 3	31 ± 2	53 ± 7	38 ± 5	34 ± 4	32 ± 4	34 ± 5	32 ± 4	33 ± 4	35 ± 3
IZED, mm										
saline	10.1 ± 0.6	10.1 ± 0.5	8.8 ± 0.6	9.7 ± 0.7	9.9 ± 0.7	10.1 ± 0.8	9.8 ± 0.7	9.7 ± 0.5	9.6 ± 0.5	9.9 ± 0.5
atenolol	9.5 ± 0.3	9.3 ± 0.4	8.2 ± 0.3	9.0 ± 0.4	9.1 ± 0.3	9.2 ± 0.3	9.0 ± 0.3	9.1 ± 0.3	9.3 ± 0.3	9.5 ± 0.3
ivabradine	10.3 ± 0.7	10.2 ± 0.7	8.4 ± 0.3	9.4 ± 0.4	9.5 ± 0.4	9.7 ± 0.4	9.7 ± 0.6	9.8 ± 0.6	9.6 ± 0.6	9.7 ± 0.6
IZ Wth, %										
saline	23 ± 4	23 ± 4	2 ± 1	11 ± 4	14 ± 4	16 ± 4	21 ± 4	18 ± 3	19 ± 3	19 ± 3
atenolol	24 ± 3	$18 \pm 4^{a,b}$	$17 \pm 4^{a,b}$	14 ± 3	17 ± 4	16 ± 4^{b}	17 ± 4^{b}	17 ± 3^{b}	17 ± 3^{b}	19 ± 3^{b}
ivabradine	23 ± 5	23 ± 5	10 ± 3^{a}	15 ± 4^{a}	20 ± 5^{a}	21 ± 5^{a}	23 ± 4	23 ± 5^{a}	25 ± 5^{a}	26 ± 5^{a}

Mean $\pm$ SEM., $n = 7$. NIZ ED: non ischaemic zone end-diastolic wall thickness; NIZ Wth: non ischaemic zone wall thickening.

[a] $P < 0.05$ significantly different from saline.

[b] $P < 0.05$ atenolol significantly different from ivabradine.

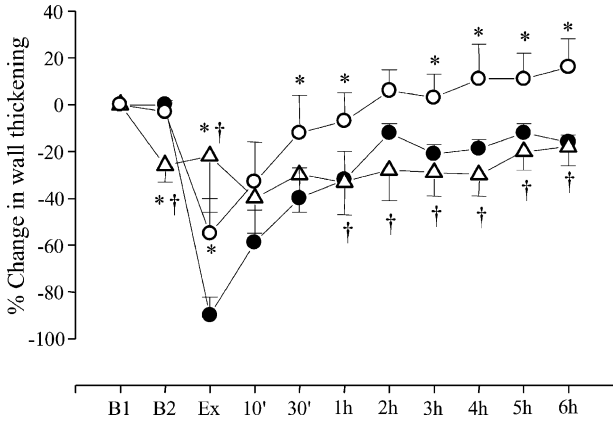

Fig. 2 Evolution of wall thickening (% change from Base 1) in the ischaemic zone as measured before (B1) and after administration (B2) of saline (full circles), ivabradine (open circles) or atenolol (open triangles) in sequence A. Recordings at baseline and during the recovery period were performed under atrial pacing at 150 beats/min. $^*P < 0.05$: significantly different from saline. $\dagger P < 0.05$: atenolol significantly different from ivabradine.

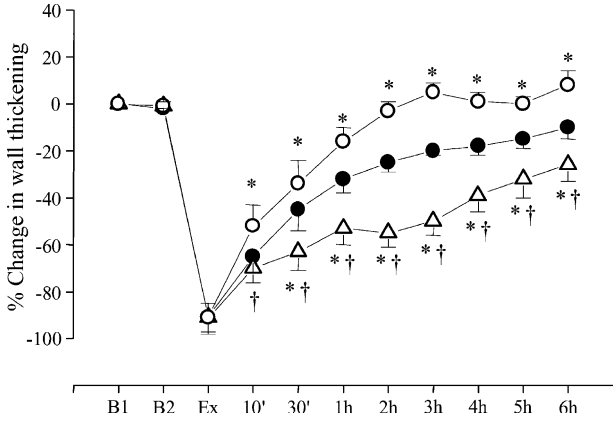

Fig. 3 Evolution of wall thickening (% change from Base 1) in the ischaemic zone as measured before (B1) and after administration (B2) of saline (full circles), ivabradine (open circles) or atenolol (open triangles) in sequence B. All recordings were performed at spontaneous heart rate. $^*P < 0.05$: significantly different from saline. $\dagger P < 0.05$: atenolol significantly different from ivabradine.

ferent and no further interaction was detected using ANOVA.

In sequence A, at Base 2 during atrial pacing, LV wall thickening was significantly reduced after administration of atenolol as compared to saline (Table 2). This parameter was not altered under ivabradine. These effects were similar at spontaneous heart rate except that LV wall thickening was significantly increased during the administration of ivabradine (data not shown). During exercise under saline, LV wall thickening increased up to $52 \pm 9\%$ from $28 \pm 2\%$ in sequence A. In the session with ivabradine, similar effects were observed but LV wall thickening was significantly limited to $34 \pm 6\%$ under atenolol ($P < 0.05$ vs saline and ivabradine). During the recovery period, LV wall thickenings measured during atrial pacing under saline and ivabradine were similar but depressed after administration of atenolol. At spontaneous heart rate, LV wall thickening was significantly greater under ivabradine as compared to saline whereas it was smaller with atenolol (e.g. at 1 h recovery, 28 ± 3 and $40 \pm 5\%$ for atenolol and ivabradine, respectively vs $35 \pm 5\%$ for saline).

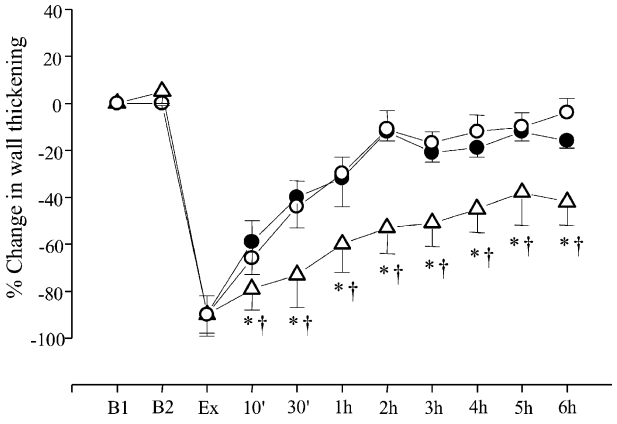

Fig. 4 Evolution of wall thickening (% change from Base 1) in the ischaemic zone as measured before (B1) and after administration (B2) of saline (full circles), ivabradine (open circles) or atenolol (open triangles) in sequence B. Recordings at baseline and during the recovery period were performed under atrial pacing at 150 beats/min. $^*P < 0.05$: significantly different from saline. $\dagger P < 0.05$: atenolol significantly different from ivabradine.

In sequence B, LV wall thickening was similar at Base 2 and during exercise in the three experimental sessions. During recovery, LV wall thickening was significantly greater under ivabradine than saline and depressed after administration of atenolol at spontaneous heart rate (e.g. at 1 h recovery, 28 ± 5 and 40 ± 4 for atenolol and ivabradine, respectively vs $35 \pm 5\%$ for saline). Under atrial pacing, LV wall thickening was similar between saline and ivabradine but still significantly reduced during the session with atenolol (Table 2).

Diastolic perfusion time

At rest, the diastolic perfusion time tended to be increased during sessions with atenolol and ivabradine as compared to saline (458 ± 39 ms, $P = 0.16$ and 478 ± 23 ms, $P = 0.06$ vs 379 ± 40 ms). This trend was reinforced during exercise under atenolol (199 ± 5 ms vs saline: 135 ± 13 ms, $P < 0.05$) and to a greater extent for ivabradine (241 ± 18 ms, $P < 0.05$ vs saline and atenolol). During the recovery period, such significant differences remained (e.g. at 2 h: 388 ± 24 ms and 411 ± 27 ms for atenolol and ivabradine, respectively vs 298 ± 25 ms for saline). The latter effect was abolished by atrial pacing for ivabradine.

LV regional myocardial blood flows

For technical reasons, measurements of RMBFs were performed in five dogs only (defective blood withdrawal in two dogs). During exercise performed under saline, transmural RMBFs increased markedly in the non-ischaemic zone, but remained unchanged in the ischaemic zone due to the coronary stenosis (Table 3).

Table 3 Regional myocardial blood flows (ml/min/g) in the ischaemic and non-ischaemic zones of the myocardium measured at baseline and during exercise

		Exercise under		
	Baseline	saline	atenolol	ivabradine
Non-ischaemic zone				
Endo	1.23 ± 0.09^{a}	2.74 ± 0.28	1.70 ± 0.14^{a}	1.62 ± 0.07^{a}
Mid	1.08 ± 0.16^{a}	2.46 ± 0.36	1.56 ± 0.27	1.41 ± 0.04^{a}
Epi	1.05 ± 0.10^{a}	2.26 ± 0.14	1.43 ± 0.11^{a}	1.36 ± 0.11^{a}
Transmural	1.12 ± 0.07^{a}	2.49 ± 0.25	1.57 ± 0.07^{a}	1.47 ± 0.06^{a}
Endo/Epi	1.18 ± 0.10	1.21 ± 0.10	1.19 ± 0.06	1.20 ± 0.05
Ischaemic zone				
Endo	1.01 ± 0.09^{a}	0.78 ± 0.04	$0.97 \pm 0.03^{a,b}$	0.92 ± 0.04^{a}
Mid	0.98 ± 0.10	0.98 ± 0.15	1.12 ± 0.21	1.06 ± 0.22
Epi	0.83 ± 0.05^{a}	1.35 ± 0.09	$1.03 \pm 0.05^{a,b}$	1.16 ± 0.06^{a}
Transmural	0.94 ± 0.08	1.04 ± 0.08	1.04 ± 0.09	1.04 ± 0.10
Endo/Epi	1.21 ± 0.03^{a}	0.58 ± 0.02	$0.94 \pm 0.04^{a,b}$	0.79 ± 0.02^{a}

Mean ± SEM., $n = 5$. Endo: endocardium; Mid: mid-myocardium; Epi: epicardium; Endo/Epi: endocardial to epicardial ratio.
[a] $P < 0.05$ significantly different from saline.
[b] $P < 0.05$ atenolol significantly different from ivabradine.

Simultaneously, the endo/epi ratio fell significantly in the ischaemic zone whereas it remained unchanged in the non-ischaemic zone.

In the ischaemic zone, during exercise in sequence A, transmural RMBFs were similar under saline, atenolol and ivabradine due to coronary stenosis. However, endocardial RMBFs and the endo/epi ratio were significantly increased under atenolol as compared to saline. Similar changes but to a smaller extent were observed in the session with ivabradine.

Discussion

Decrease in heart rate is one of the goals to achieve for the treatment of ischaemic heart disease. In this setting, *If* inhibitors form a novel class of heart rate reducing agents which may be useful to achieve this goal. The present results confirm our previous results, i.e., that heart rate reduction induced by an I*f* inhibitor is of major importance during exercise induced myocardial ischaemia and subsequent stunning.[9] This study demonstrates that the negative inotropic effect of a β-blocker such as atenolol modulates the cardioprotection afforded by heart rate reduction, either positively or negatively, depending on the time of administration. Indeed, heart rate reduction provides a powerful anti-ischaemic effect and additional negative inotropism afforded by atenolol tends to enhance this effect. Conversely, administered after exercise-induced ischaemia, i.e., when the myocardium is stunned, regional contractility is improved by selective heart rate reduction with ivabradine but is dramatically deteriorated by the negative inotropism of atenolol.

The experimental model used in this study enabled to investigate the anti-ischaemic effects associated with the reductions respectively in heart rate and in LV inotropism as these parameters were strongly and physiologically enhanced during treadmill exercise. The combination of an exercise and a coronary artery stenosis induced a severe regional imbalance between myocardial metabolic demand and oxygen supply.[9,14,17] This resulted in a strong decrease in regional contractility in the ischaemic zone and a subsequent myocardial stunning. Importantly, in this model, repetition of exercises does not induce any late preconditioning-like effect as all experiments were performed at least 5 days apart, a delay after which any potential late preconditioning has vanished.[18] Furthermore, late preconditioning against stunning does not occur after exercise-induced ischaemia in conscious dogs.[14]

In this study, selective heart rate reduction during the ischaemic insult induced by exercise clearly reduced regional myocardial contractile dysfunction in agreement with previous studies.[8,9,19] Recently, we demonstrated that the beneficial effect of ivabradine was due to its negative chronotropic property as atrial pacing abolished its anti-ischaemic effects.[9] This cardioprotection appears likely due to an enhanced diastolic perfusion time, an increased subendocardial perfusion and as previously reported,[16,20] a decreased myocardial oxygen demand. In animal models using ameroïds, atenolol provided also a strong anti-ischaemic effect as previously reported.[2,7] In our experimental conditions, in addition to the reduction in heart rate, the negative inotropic effect of atenolol tends to provide some anti-ischaemic effect during exercise. This could be due to a more favourable balance between oxygen demand and supply and we previously reported that atenolol decreases markedly more myocardial oxygen consumption than ivabradine during exercise in the normal heart.[16] In addition, with β-blockade, the auto-regulated increase in vascular resistance in the distal bed improves subendocardial perfusion.[6] Finally, as transmural myocardial blood flows were similar among exercises, differences in blood supply levels due to different coronary stenosis levels cannot explain these differences between drugs.

One of the main results of this study is that opposite effects were observed depending on the time of administration of ivabradine and atenolol. As heart rate reduction can per se modify LV wall thickening,[9] we thought that this factor should be taken into account for the interpretation of the cardioprotective effect of ivabradine and atenolol. Accordingly in sequence A, only values measured under atrial pacing (150 beats/min) at baseline and during the recovery period were analyzed. In these conditions, administration of ivabradine prior to ischaemia dramatically reduced the severity of myocardial stunning during recovery. In contrast, LV wall thickening measured under atenolol was altered at baseline and remained depressed throughout the 6 h of the recovery period and similar to that under saline. This means that the negative inotropic effect of atenolol generated dual but opposite effects, i.e., a cardioprotective one during ischaemia and a deleterious one during myocardial stunning. In fact, the negative inotropism of atenolol overrides its anti-ischaemic effect and further abrogates the cardioprotection afforded by heart rate reduction on regional myocardial contractility. In contrast, selective heart rate reduction with ivabradine attenuated myocardial dysfunction during ischaemia and further extended this effect to the protection against myocardial stunning.

This crucial role of the time of administration is also illustrated in sequence B when drugs were given at exercise completion, i.e., under conditions independent from any previous anti-ischaemic effect. As we previously demonstrated,[9] selective heart rate reduction induced after ischaemia by ivabradine resulted in a significant enhancement of regional wall thickening as compared to saline. As this effect was abolished by atrial pacing, it appears solely due to heart rate reduction and not related to any direct action on the cardiomyocyte of the ischaemic zone, i.e., not related to a positive inotropic effect. It can be hypothesized that heart rate reduction improves the diastolic time for LV filling,[16] increases the end-diastolic LV volume and finally enhances the LV regional contractility through a Frank–Starling mechanism. Indeed, the enhancement of myocardial contractility was also observed in the non-ischaemic zone and ivabradine increased LV end-diastolic diameter in the normal heart (data not shown). In contrast, atenolol clearly deteriorated LV wall thickening of the stunned myocardium as a consequence of its negative inotropic effect.

Some limitations of this study should be addressed. Only one dose of atenolol was used in this study and therefore it is possible that different results would be observed with other beta-blockers eliciting other pharmacological profile or with smaller doses of atenolol. These findings should also not be extended to other situations with chronic LV dysfunction and likely different pathophysiological mechanisms, as beneficial effects with small doses of β-blockers are well recognized in the setting of chronic heart failure.[21] Finally from a statistical point of view, since the cross-over design is unbalanced in this study, the treatment effects cannot be unambiguously attributed to the treatment per se and could also include period and/or wash-out effects. In conclusion, this study highlights the fundamental role of reducing heart rate in the protection of the ischaemic and stunned myocardium induced by exercise associated with a coronary stenosis. In the case of atenolol, despite its negative inotropic effect which tends to enhance the anti-ischaemic effect afforded by heart rate reduction during ischaemia, it aggravates the LV systolic dysfunction of the stunned myocardium. In contrast, selective heart rate reduction with ivabradine not only affords cardioprotection during exercise-induced ischaemia but also strongly enhances regional contractility of the stunned myocardium. Although the recovery from myocardial ischaemia depends from many more factors including LV function at baseline, this might have important clinical implications during LV systolic dysfunctions, especially in the setting of chronic and repeated myocardial stunning as ivabradine has been demonstrated to be a potent anti-ischaemic agent in humans.[22]

Acknowledgements

Dr Patrice Colin was supported by the Société Française de Pharmacologie. We thank Dr F. Mahlberg, Dr J. P. Vilaine and Dr G. Lerebourg from Laboratoires Servier for fruitful discussions during the elaboration of this manuscript. The authors are greatly indebted to Dr J. X. Mazoit for statistical advises. We wish also to thank Alain Bizé and Stéphane Bloquet for their excellent technical assistance.

References

1. Hjalmarson A, Gilpin EA, Kjekshus J et al. Influence of heart rate on mortality after acute myocardial infarction. *Am J Cardiol* 1990;**65**:547–53.
2. Matsuzaki M, Patritti J, Tajimi T et al. Effects of beta-blockade on regional myocardial flow and function during exercise. *Am J Physiol* 1984;**247**:H52–60.
3. Kloner RA, Kirshenbaum J, Lange R et al. Experimental and clinical observations on the efficacy of esmolol in myocardial ischemia. *Am J Cardiol* 1985;**56**:40F–8F.
4. Przyklenk K, Kloner RA. Is 'stunned myocardium' a protective mechanism? Effect of acute recruitment and acute β-blockade on recovery of contractile function and high-energy phosphate stores at 1 day post-reperfusion. *Am Heart J* 1989;**118**:480–9.
5. Al-Wathiqui MH, Farber N, Pelc L et al. Improvement in functional recovery of stunned canine myocardium by long-term pretreatment with oral propranolol. *Am Heart J* 1989;**117**:791–8.
6. Buck JD, Hardman HF, Warltier DC et al. Changes in ischemic blood flow distribution and dynamic severity of a coronary stenosis induced by beta blockade in the canine heart. *Circulation* 1981;**64**:708–15.
7. Guth BD, Heusch G, Seitelberger R et al. Mechanism of beneficial effect of beta-adrenergic blockade on exercise-induced myocardial ischemia in conscious dogs. *Circ Res* 1987;**60**:738–46.
8. Guth BD, Heusch G, Steitelberger R et al. Elimination of exercise-induced regional myocardial dysfunction by a bradycardic agent in dogs with chronic coronary stenosis. *Circulation* 1987;**75**:661–9.
9. Monnet X, Ghaleb B, Colin P et al. Effects of heart rate reduction with ivabradine on exercise-induced myocardial ischemia and stunning. *J Pharmacol Exp Ther* 2001;**299**:1133–9.
10. Thollon C, Cambarrat C, Vian J et al. Electrophysiological effects of S 16 257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: comparison with UL-FS 49. *Br J Pharmacol* 1994;**112**:37–42.

11. Bois P, Bescond J, Renaudon B et al. Mode of action of bradycardic agent, S 16 257, on ionic currents of rabbit sino-atrial node cells. *Br J Pharmacol* 1996;**118**:1051—7.
12. Thollon C, Bidouard JP, Cambarrat C et al. Stereospecific in vitro and in vivo effects of the new sinus node inhibitor (+)-S 16257. *Eur J Pharmacol* 1997;**339**:43—51.
13. Simon L, Ghaleb B, Puybasset L et al. Coronary hemodynamic effects of S 16 257, a new bradycardic agent, in resting and exercising conscious dogs. *J Pharmacol Exp Ther* 1995;**275**:659—66.
14. Parent de Curzon O, Ghaleb B, Giudicelli JF et al. Myocardial stunning in exercise-induced ischemia in dogs: lack of late preconditioning. *Am J Physiol* 2001;**280**:H302—10.
15. Ghaleb B, Bea ML, Dubois-Rande JL et al. Endothelial modulation of beta-adrenergic dilation of large coronary arteries in conscious dogs. *Circulation* 1995;**92**:2627—35.
16. Colin P, Ghaleb B, Monnet X et al. Contributions of heart rate and contractility to myocardial oxygen balance during exercise. *Am J Physiol* 2003;**284**:H676—82.
17. Homans DC, Sublett E, Dai XZ et al. Persistence of regional left ventricular dysfunction after exercise-induced myocardial ischemia. *J Clin Invest* 1986;**77**:66—73.
18. Tang XL, Qiu Y, Park SW et al. Time course of late preconditioning against myocardial stunning in conscious pigs. *Circ Res* 1996;**79**:424—34.
19. Dämmgen JW, Lamping KA, Gross GJ. Actions of two new bradycardic agents, AQ-AH 208 and UL-FS 49, on ischemic myocardial perfusion and function. *J Cardiovasc Pharmacol* 1985;**7**: 71—9.
20. Indolfi C, Guth BD, Miura T et al. Mechanisms of improved ischemic regional dysfunction by bradycardia: studies on UL FS 49 in swine. *Circulation* 1989;**80**:983—93.
21. Gheorghiade M, Colucci WS, Swedberg K. Blockers in chronic heart failure. *Circulation* 2003;**107**:1570—5.
22. Ishikawa K, Sugawara D, Wang XP et al. Heme oxygenase-1 inhibits atherosclerotic lesion formation in LDL-receptor knockout mice. *Circ Res* 2001;**88**:506—12.