Improvement of regional myocardial blood flow and function and reduction of infarct size with ivabradine: protection beyond heart rate reduction

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Aims
Effects of the bradycardic agent ivabradine on regional blood flow, contractile function, and infarct size were studied in a pig model of myocardial ischaemia/reperfusion. Heart rate reduction by β-blockade is associated with negative inotropism and unmasked α-adrenergic coronary vasoconstriction. Ivabradine is the only available bradycardic agent for clinical use.

Methods and results
Anaesthetized pigs were subjected to 90 min controlled left anterior descending coronary artery hypoperfusion and 120 min reperfusion. Regional blood flow was measured with microspheres, regional function with sonomicrometry, and infarct size with triphenyl tetrazolium chloride staining. Pigs received placebo or ivabradine (0.6 mg/kg i.v.) before or during ischaemia or before reperfusion, respectively.

Pre-treatment with ivabradine reduced infarct size from 35 ± 4 (SEM) to 19 ± 4% of area at risk (AAR). Ivabradine 15–20 min after the onset of ischaemia increased regional myocardial blood flow from 2.12 ± 0.31 to 3.55 ± 0.56 mL/beat/g and systolic wall thickening from 6.7 ± 1.0 to 16.3 ± 3.0%; infarct size was reduced from 12 ± 4 to 2 ± 1% of AAR. Ivabradine 5 min before reperfusion still reduced infarct size from 36 ± 4 to 21 ± 5% of AAR. The benefit of ivabradine on flow and function was eliminated by atrial pacing, but part of the reduction of infarct size by ivabradine was not.

Conclusion
Ivabradine’s protection goes beyond heart rate reduction.

Keywords
Bradycardic agent • Myocardial ischaemia • Myocardial infarction • Reperfusion • Heart rate

Introduction
The detrimental effects of tachycardia and the beneficial effects of heart rate reduction in myocardial ischaemia are well established.1,2

β-Blockade reduces heart rate and attenuates myocardial ischaemia, resulting in improved blood flow and contractile function3,4 and reduced infarct size.5,6 However, β-blockade has negative inotropic actions which are unwanted when ischaemia impairs ventricular function. Also, when heart rate reduction is eliminated by atrial pacing, β-blockade exerts negative effects on regional blood flow and function,7 largely through unmasked α-adrenergic coronary vasoconstriction.8–10 These undesired effects of β-blockade have prompted the development of drugs which reduce heart rate more selectively.11 Several more selective bradycardic agents which act through inhibition of the I_{f}-channel in the sinus node have proven benefit in models of myocardial ischaemia/reperfusion in terms of improved blood flow and function12–16 and reduced infarct size.6,17

The only selective bradycardic agent which is currently available for clinical use is ivabradine, and ivabradine attenuates exercise-induced myocardial ischaemia in patients with chronic stable angina.18 Ivabradine reduces heart rate selectively by inhibition of the I_{f}-channel in the sinus node.11

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Data for ivabradine’s effects on the relationship of regional blood flow and function and on infarct size are not available. We have, therefore, used our established pig model of regional ischaemia/reperfusion$^{6,19}$ and studied the effects of ivabradine on blood flow (microspheres), contractile function (sonomicrometry), and infarct size (TTC staining). The role of heart rate per se was addressed by eliminating the heart rate reduction with ivabradine by atrial pacing. The data were then integrated into a conceptual framework of myocardial ischaemia in terms of supply and demand.

### Methods

The experimental protocols were approved by the Bioethical Committee of the district of Düsseldorf.

#### Experimental preparation

As described previously$^{6,19}$ 69 Göttinger minipigs (20–40 kg) were sedated using ketamine hydrochloride (1 g intramuscularly) and anaesthetized with thiopental (500 mg intravenously). Through a cervical incision, the trachea was intubated and connected to a respirator (Dräger, Lubeck, Germany). Anaesthesia was maintained using enfurane (1–1.5%) with an oxygen/nitrous oxide mixture (40:60%). The common carotid arteries were cannulated to measure arterial pressure and to supply blood to the extracorporeal circuit. The jugular veins were cannulated for volume replacement. A left lateral thoracotomy was performed and the pericardium opened. A micromanometer (P7, Königsberg Instr., Pasadena, CA, USA) was placed in the left ventricle through the apex together with a saline-filled polyethylene catheter (for calibration). Ultrasonic dimension gauges were implanted in the left ventricular (LV) myocardium to measure the thickness of the anterior and posterior (control) wall. The left anterior descending (LAD) coronary artery was dissected over a distance of 1.5 cm, ligated, cannulated, and perfused from an extracorporeal circuit. Pigs were anticoagulated with 20,000 IU sodium heparin and additional doses of 10,000 IU at 2 h intervals. The system included a roller pump, windkessel, and side-port for the injection of radiolabelled microspheres. Coronary arterial pressure was measured from the sidearm of a polyethylene T-connector (pvb Medizintechnik, Kirchseen, Germany). Minimal coronary arterial pressure was held above 75 mmHg by adjusting the roller pump to avoid hypoperfusion prior to ischaemia. Heart rate could be controlled by left atrial pacing (Hugo Sachs Elektronik Type 215/T, Hugstetten, Germany).

#### Regional myocardial blood flow

Radiolabelled microspheres (15 μm in diameter; $^{141}$Ce, $^{114}$In, $^{51}$Cr, $^{103}$Ru, $^{95}$Nb, or $^{46}$Sc; NEN-DuPont, Boston, USA) were injected into the coronary perfusion circuit to determine regional blood flow (model 5912, Gammaszint BF 5300 Packard, Germany). Transmural blood flow, when related to infarct size, was calculated as the mean regional myocardial blood flow in all tissue samples of the area at risk (AAR). Transmural blood flow, when related to regional myocardial function, was calculated as the average of endocardial, midmyocardial, and epicardial blood flow in the crystal area. Blood flow is presented as flow per minute and, after normalization for heart rate, as flow per beat.

#### Regional contractile function

In addition to systolic wall thickening, the sum of the instantaneous LV pressure development—wall thickening product during systole was determined.$^{20}$ To estimate cumulative regional work during 90 min ischaemia, this sum of systolic pressure development—wall thickening product was determined beat-by-beat and multiplied with the number of beats.

### Infarct size

At the end of each study, the heart was sectioned from base to apex into five transverse slices in a plane parallel to the atrioventricular groove. Slices were immersed in 0.09 mol/L sodium phosphate buffer containing 1.0% triphenyl tetrazolium chloride (Sigma-Aldrich Chemie GmbH, Munich, Germany) and 8% debran for 20 min at 37°C. The amount of infarcted tissue is expressed as percent of the AAR, as defined by the microspheres technique, i.e. a regional myocardial blood flow greater than 0.25 mL/min/g at baseline.

#### Experimental protocols

Protocol 1

This protocol served to study the effect of ivabradine pre-treatment on infarct size under rigorous circumstances. Therefore, coronary inflow was matched in groups 1–3 and maintained constant. In the pig, collateral blood flow is negligible and, therefore, the severity of myocardial ischaemia can be controlled through coronary inflow. Nevertheless, the amount of residual blood flow remains a major determinant of infarct size, and infarct size was, therefore, plotted as a function of residual blood flow.

- **Group 1** ($n=8$). Following baseline measurements of haemodynamics, regional blood flow, and function, ivabradine (0.6 mg/kg) was given intravenously. In preliminary experiments, this dose was found to reduce heart rate by 20–30 b.p.m. After reaching a steady state, measurements of haemodynamics, regional blood flow and function were repeated. Coronary inflow was then reduced to 15% of baseline and maintained constant. At 5–10 min ischaemia measurements of haemodynamics, regional blood flow and function were again repeated. After 90 min ischaemia, the myocardium was reperfused for 2 h before infarct size was determined.

- **Group 2** ($n=8$). The protocol was identical to that of group 1, except that the infusion of ivabradine was replaced by saline.

- **Group 3** ($n=6$). The protocol was identical to that of group 1, except that heart rate was controlled by atrial pacing slightly above the spontaneous rate.

Protocol 2

This protocol served to study the effect of ivabradine on infarct size when treatment was started after the onset of ischaemia. This protocol also permitted to study the effects of ivabradine on the relationship between regional blood flow and function. Therefore, to permit changes in blood flow, coronary perfusion pressure was maintained constant during ischaemia. The degree of ischaemia was set somewhat less severe than in protocol 1 to retain a positive, though minimal contractile function during ischaemia even with placebo. This somewhat milder degree of ischaemia also permitted to extend the range of infarct size vs. residual blood flow.

- **Group 4** ($n=9$). Following baseline measurements of haemodynamics, regional blood flow and function, coronary inflow was reduced to achieve a 85% reduction in regional function. At 5–10 min ischaemia measurements of haemodynamics, regional blood flow and function were repeated, and coronary perfusion pressure was maintained constant. Ivabradine (0.6 mg/kg) was then given intravenously. At 15–20 min ischaemia, at a new steady state, measurements of haemodynamics, regional blood flow and function were again repeated. After 90 min ischaemia, the myocardium was reperfused for 2 h before infarct size was determined.
Group 5 (n = 10). The protocol was identical to that of group 3, except that the infusion of ivabradine was replaced by saline.

Group 6 (n = 6). The protocol was identical to that of group 3, except that heart rate was controlled by atrial pacing slightly above the spontaneous rate.

Protocol 3
This protocol served to study the effects of ivabradine on reperfusion injury. To this effect, measurements of regional myocardial blood flow and systolic wall thickening at 10 min reperfusion were also performed.

Group 7 (n = 7). The protocol was identical to that of group 1, except that ivabradine was given 5 min before reperfusion.

Group 8 (n = 7). The protocol was identical to that of group 7, except that the infusion of ivabradine was replaced by saline.

Group 9 (n = 8). The protocol was identical to that of group 7, except that heart rate was controlled by atrial pacing slightly above the spontaneous rate.

Statistics
Data are mean ± SEM. Haemodynamics were analysed by two-way ANOVA for repeated measurements. Areas at risk and infarct sizes were analysed by one-way ANOVA. When a significant difference was detected, individual mean values were compared by post hoc tests (LSD).

Linear regression analyses between regional blood flow in the AAR and infarct size and between regional blood flow and systolic wall thickening (data points at baseline and during 5–10 min ischaemia) in the crystal area were performed and compared by ANCOVA.

Two-dimensional analyses of systolic wall thickening vs. regional blood flow per minute and per beat, respectively, in the crystal area were performed by Hotelling’s multivariate t-test for two variables. Differences were considered significant at the level of P < 0.05.

Results
Ventricular extrasystoles occurred during ischaemia and early reperfusion but were not different between groups.

Protocol 1
Baseline haemodynamics, regional blood flow, and contractile function were well matched between groups 1, 2, and 3 (Table 1). Ivabradine initially reduced heart rate by an average of 26 b.p.m.; this effect waned somewhat during the following 90 min ischaemia, but remained significant. Heart rate reduction by ivabradine was initially associated with increased systolic wall thickening, but no other difference in systemic and regional haemodynamics between groups 1, 2, and 3 was observed. The cumulative work index was 153 ± 37 mmHg mm in group 1 vs. 119 ± 62 mmHg mm in group 2 vs. 45 ± 3 ± 3 mmHg mm in group 3 (NS). AAR was 50 ± 1% of LV in group 1, 46 ± 2% in group 2, and 44 ± 3% in group 3 (NS). Infarct size was 19 ± 4% of AAR in group 1, 35 ± 4% in group 2 (P < 0.05 vs. group 1), and 25 ± 5% in group 3. The relationship between blood flow and infarct size was significantly displaced downwards in group 1 when compared with that in group 2; in group 3, the relationship was between those of groups 1 and 2 and significantly different from that in group 2 (Figure 1).

Protocol 2
Haemodynamics, regional blood flow, and contractile function were well matched between groups 4, 5, and 6 at baseline and during early ischaemia (Table 2). With administration of ivabradine during ischaemia in group 4, heart rate was decreased and contractile function was improved. Regional blood flow per minute tended to increase after ivabradine; regional blood flow per beat was significantly increased. There was no difference in haemodynamics, regional blood flow, and contractile function between groups 5 and 6. The relationships between blood flow and function, comprising data at baseline and during 5–10 min ischaemia, were not different between groups 4, 5, and 6. In a two-dimensional analysis of regional blood flow and systolic wall thickening, ivabradine improved flow and function along a consistent flow–function relationship, no matter whether flow was calculated per minute or per beat (Figure 2).

The cumulative work index during 90 min ischaemia was 517 ± 83 mmHg mm in groups 4, 5, and 6; 394 ± 53 mmHg mm in groups 4, 5, and 6, respectively (NS). AAR was 45 ± 2, 49 ± 2, and 46 ± 3% of LV in groups 4, 5, and 6, respectively (NS). Infarct size in group 4 was 2 ± 1% of AAR and less than that in groups 5 (12 ± 4%, P < 0.05) and 6 (5 ± 2%, NS). The relationship between blood flow and infarct size in group 4 was significantly displaced downwards when compared with that in group 5 (Figure 3). The regression line in group 6 was somewhere in between and not significantly different from those in groups 4 and 5.

Protocol 3
Baseline haemodynamics, regional blood flow, and contractile function were well matched between groups 7, 8, and 9 (Table 3). The cumulative work index during 90 min ischaemia was 20 ± 39 mmHg mm in groups 7, 8, and 9, respectively (NS). Ivabradine administered 5 min before reperfusion reduced heart rate by an average of 22 b.p.m. AAR was 45 ± 3, 46 ± 3, and 42 ± 2% of LV in groups 7, 8, and 9, respectively (NS). Infarct size was 21 ± 5% of AAR in group 7, 36 ± 4% in group 8 (P < 0.05 vs. groups 7 and 9), and 23 ± 4% in group 9. The relationships between blood flow and infarct size were significantly displaced downwards in groups 7 and 9 when compared with that in group 8 (Figure 4).

Discussion
The major results of the present study are:

1. Heart rate reduction by ivabradine increases ischaemic regional blood flow and contractile function proportionately.

2. Ivabradine reduces infarct size even when given only after the onset of ischaemia or just before reperfusion.

3. There is no residual negative effect of ivabradine on flow and function when heart rate reduction is eliminated by atrial pacing. The beneficial effects of ivabradine, when given before or during ischaemia, on infarct size are attenuated, but not eliminated by atrial pacing.
Table 1  Haemodynamics, contractile function, and regional myocardial blood flow of groups 1, 2, and 3

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (1/min)</th>
<th>LVPmax (mmHg)</th>
<th>dP/dtmax (mmHg/s)</th>
<th>CAPmean (mmHg)</th>
<th>CBFmean (mL/min)</th>
<th>WTpost (%)</th>
<th>WTant (%)</th>
<th>LOOPaw (mmHg mm)</th>
<th>RMBF (mL/min/g)</th>
<th>RMBF (mL/beat/g)</th>
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<tr>
<td>Baseline</td>
<td>91 ± 4</td>
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<td>3.9 ± 0.7*</td>
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<td>74 ± 3*</td>
<td>78 ± 2*</td>
<td>1109 ± 61*</td>
<td>27 ± 1*</td>
<td>3.9 ± 0.7*</td>
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<tr>
<td>Baseline</td>
<td>93 ± 4</td>
<td>93 ± 2</td>
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<td>77 ± 1*</td>
<td>1055 ± 65*</td>
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<td>19.3 ± 3.8</td>
<td>0.5 ± 2.1*</td>
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<td>80 ± 1*</td>
<td>1109 ± 62*</td>
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<td>1181 ± 116*</td>
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<tr>
<td>Baseline</td>
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<td>98 ± 3</td>
<td>1502 ± 54</td>
<td>116 ± 2</td>
<td>23.8 ± 4.0</td>
<td>23.4 ± 4.7</td>
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<td>−3.6 ± 1.0</td>
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<td>−0.6 ± 0.7*</td>
<td>4 ± 3*</td>
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</table>

Isch5/30/60/90: 5/30/60/90 min after the onset of ischaemia.
HR, heart rate; LVPmax, maximal left ventricular pressure; dP/dtmax, maximal rate of rise of left ventricular pressure; CAPmean, mean coronary perfusion pressure; CBFmean, mean coronary blood flow; WTpost, systolic wall thickening in the posterior wall; WTant, systolic wall thickening in the anterior wall; LOOPaw, instantaneous LV pressure-wall thickening product during systole in the anterior wall; RMBF, transmural blood flow in the area at risk either expressed per time or per beat.
*P < 0.05 vs. baseline (two-way ANOVA for repeated measures and Fisher's LSD post hoc tests).
**P < 0.05 vs. Group 2 (two-way ANOVA for repeated measures and Fisher's LSD post hoc tests).
***P < 0.05 vs. Group 3 (two-way ANOVA for repeated measures and Fisher's LSD post hoc tests).
In normal myocardium, increased heart rate increases myocardial oxygen consumption, and the increased myocardial oxygen consumption is matched by increased coronary blood flow through metabolic coronary vasodilation. Yet, coronary blood flow per cardiac cycle is decreased because the duration of diastole is reduced overproportionately at increased heart rate. Therefore, a slight increase in myocardial oxygen extraction helps to match oxygen supply to oxygen demand during tachycardia.

When coronary dilator reserve is compromised and finally exhausted distal to severe coronary stenoses, metabolic dilation is no longer possible and the effects of reduced diastolic duration prevail, such that coronary blood flow is reduced at increased heart rate. In the setting of regional ischaemia, both metabolic vasodilation in normal myocardium and reduced diastolic duration in post-stenotic myocardium act in concert to redistribute blood flow away from the post-stenotic myocardium, particularly at the expense of subendocardial layers which depend most on diastolic duration.

Pharmacological heart rate reduction and myocardial ischaemia

Attenuation of tachycardia during exercise-induced myocardial ischaemia by β-blockade reverses the above unfavourable blood flow redistribution. The more favourable blood flow distribution towards the ischaemic myocardium by β-blockade manifests in improved regional contractile function and ultimately in reduced infarct size. However, when the attenuation of exercise-related tachycardia by β-blockade is eliminated by atrial pacing, a residual negative effect on ischaemic regional blood flow and contractile function becomes apparent, most likely caused by unmasked α-adrenergic coronary vasoconstriction. In addition, β-blockers exert negative inotropic actions on normal myocardium and in consequence on global LV function. These unwanted effects of β-blockers have prompted the development of drugs which reduce heart rate more selectively. A number of bradycardic agents have proven benefit in terms of ischaemic regional blood flow, contractile function, and infarct size in models of ischaemia/reperfusion. Ivabradine is the only bradycardic agent which is available for clinical use in patients with chronic stable angina. Ivabradine reduces myocardial oxygen consumption and prolongs diastolic duration in normal myocardium. Ivabradine attenuates ischaemic and post-ischaemic contractile dysfunction with exercise-induced ischaemia in dogs and pigs. Ivabradine does not unmask α-adrenergic coronary vasoconstriction in epicardial arteries. These favourable effects of ivabradine were confirmed in the present study and extended to demonstrate a shift of both ischaemic myocardial blood flow and systolic wall thickening along a more or less linear flow–function relationship as characterized by others before. The observed shift of ischaemic myocardial blood flow and systolic wall thickening was reversed but not negatively affected when heart rate reduction was eliminated by atrial pacing (Figure 2). These data provide a mechanism for the clinical study where ivabradine attenuated exercise-induced ischaemia in patients with chronic stable angina.
Table 2 Haemodynamics, contractile function, and regional myocardial blood flow of groups 4, 5, and 6

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (1/min)</th>
<th>LVPMax (mmHg)</th>
<th>DPdmax (mmHg/s)</th>
<th>CAPmean (mmHg)</th>
<th>CBFmean (mL/min)</th>
<th>WTpost (%)</th>
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<tr>
<td>Baseline</td>
<td>91 ± 3</td>
<td>101 ± 4</td>
<td>1548 ± 65</td>
<td>119 ± 4</td>
<td>22 ± 2</td>
<td>17.5 ± 1.2</td>
<td>44.6 ± 2.9</td>
<td>341 ± 25</td>
<td>0.564 ± 0.031</td>
<td>6.16 ± 0.40</td>
</tr>
<tr>
<td>Isch5–10</td>
<td>92 ± 3</td>
<td>93 ± 4</td>
<td>1253 ± 68</td>
<td>33 ± 3</td>
<td>8 ± 1</td>
<td>18.0 ± 1.5</td>
<td>6.7 ± 1.0</td>
<td>43 ± 8</td>
<td>0.190 ± 0.023</td>
<td>2.10 ± 0.28</td>
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<tr>
<td>Isch15–20</td>
<td>65 ± 3*+</td>
<td>90 ± 3*+</td>
<td>1175 ± 66</td>
<td>32 ± 3*+</td>
<td>9 ± 1*+</td>
<td>20.4 ± 2.1</td>
<td>16.3 ± 3.0*+</td>
<td>82 ± 15*+</td>
<td>0.227 ± 0.035*</td>
<td>3.55 ± 0.56*+</td>
</tr>
<tr>
<td>Isch30</td>
<td>70 ± 4*+</td>
<td>90 ± 3*+</td>
<td>1185 ± 74</td>
<td>33 ± 3*+</td>
<td>9 ± 1*+</td>
<td>21.0 ± 1.8</td>
<td>15.5 ± 3.9*+</td>
<td>85 ± 19*+</td>
<td>0.595 ± 0.038</td>
<td>4.34 ± 0.65</td>
</tr>
<tr>
<td>Isch60</td>
<td>71 ± 4*+</td>
<td>91 ± 3*+</td>
<td>1232 ± 73</td>
<td>32 ± 3*+</td>
<td>9 ± 1*+</td>
<td>21.7 ± 2.0</td>
<td>15.0 ± 3.2*+</td>
<td>82 ± 17*+</td>
<td>0.671 ± 0.042</td>
<td>4.92 ± 0.71</td>
</tr>
<tr>
<td>Isch90</td>
<td>74 ± 4*+</td>
<td>90 ± 3*+</td>
<td>1219 ± 71</td>
<td>32 ± 3*+</td>
<td>9 ± 1*+</td>
<td>21.6 ± 1.9</td>
<td>13.3 ± 2.2*+</td>
<td>74 ± 15*+</td>
<td>0.748 ± 0.047</td>
<td>5.48 ± 0.77</td>
</tr>
</tbody>
</table>

| **Group 5**   |            |                |                 |                |                  |            |             |                  |                |                 |
| Baseline      | 93 ± 4     | 104 ± 3        | 1460 ± 47      | 118 ± 3        | 28 ± 4           | 18.9 ± 1.9 | 43.3 ± 3.5  | 355 ± 21        | 0.557 ± 0.046   | 6.08 ± 0.49     |
| Isch5–10      | 94 ± 4     | 96 ± 3*        | 1197 ± 34      | 36 ± 2*        | 11 ± 2*          | 19.6 ± 2.4 | 5.1 ± 1.2   | 40 ± 9*         | 0.198 ± 0.031*  | 2.16 ± 0.32*    |
| Isch15–20     | 94 ± 5     | 96 ± 2*        | 1205 ± 33      | 36 ± 2*        | 11 ± 2*          | 19.7 ± 2.4 | 3.3 ± 1.2   | 29 ± 9*         | 0.195 ± 0.029*  | 2.12 ± 0.31*    |
| Isch30        | 93 ± 5     | 96 ± 3         | 1211 ± 30      | 36 ± 2*        | 11 ± 2*          | 20.0 ± 2.5 | 3.3 ± 1.2   | 31 ± 11*        | 0.243 ± 0.036   | 2.68 ± 0.37*    |
| Isch60        | 95 ± 4     | 96 ± 3*        | 1278 ± 36      | 33 ± 2*        | 11 ± 2*          | 19.9 ± 2.4 | 4.6 ± 1.1*  | 38 ± 10*        | 0.288 ± 0.039   | 3.21 ± 0.41*    |
| Isch90        | 99 ± 4     | 96 ± 3*        | 1294 ± 39      | 34 ± 2*        | 11 ± 2*          | 19.4 ± 2.9 | 4.8 ± 1.4*  | 47 ± 14*        | 0.333 ± 0.045   | 3.75 ± 0.51*    |

| **Group 6**   |            |                |                 |                |                  |            |             |                  |                |                 |
| Baseline      | 96 ± 4     | 103 ± 3        | 1364 ± 55      | 121 ± 2        | 25 ± 3           | 11.7 ± 1.5*| 38.2 ± 4.7  | 311 ± 33        | 0.566 ± 0.045   | 5.94 ± 0.51     |
| Isch5–10      | 96 ± 4     | 103 ± 2        | 1135 ± 30      | 34 ± 3*        | 9 ± 2*           | 12.5 ± 1.7*| 5.0 ± 0.9   | 32 ± 4*         | 0.181 ± 0.031*  | 1.87 ± 0.31*    |
| Isch15–20     | 96 ± 4     | 102 ± 3        | 1167 ± 21      | 36 ± 3*        | 9 ± 2*           | 11.7 ± 1.9*| 3.0 ± 0.9   | 23 ± 6*         | 0.173 ± 0.028*  | 1.78 ± 0.38*    |
| Isch30        | 96 ± 4     | 98 ± 3         | 1174 ± 16      | 35 ± 3*        | 9 ± 2*           | 12.8 ± 2.2*| 2.1 ± 1.2*  | 27 ± 9*         | 0.210 ± 0.039   | 2.31 ± 0.53*    |
| Isch60        | 96 ± 4     | 96 ± 3         | 1245 ± 18      | 33 ± 3*        | 9 ± 2*           | 13.9 ± 2.6 | 5.4 ± 1.6*  | 49 ± 11*        | 0.257 ± 0.044   | 2.88 ± 0.55*    |
| Isch90        | 96 ± 4     | 96 ± 2         | 1244 ± 36      | 33 ± 3*        | 9 ± 2*           | 13.5 ± 2.4 | 6.3 ± 1.6*  | 56 ± 9*         | 0.293 ± 0.048   | 3.41 ± 0.63*    |

Isch5–10/15–20/30/60/90: 5–10/15–20/30/60/90 min after the onset of ischaemia.
HR, heart rate; LVPMax, maximal left ventricular pressure; DPdmax, maximal rate of rise of left ventricular pressure; CAPmean, mean coronary perfusion pressure; CBFmean, mean coronary blood flow; WTpost, systolic wall thickening in the posterior wall; WTant, systolic wall thickening in the anterior wall; LOOPaw, instantaneous LV pressure development—wall thickening product during systole in the anterior wall; RMBF, transmural blood flow in the area at risk either expressed per time or per beat.

*P < 0.05 vs. baseline (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).
†P < 0.05 vs. Isch5–10 (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).
‡P < 0.05 vs. Group 5 (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).
§P < 0.05 vs. Group 6 (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).
Figure 2 Anterior systolic wall thickening as a function of regional blood flow (A) expressed as blood flow per minute and (B) expressed as blood flow per beat. In both plots, ivabradine improves blood flow and systolic wall thickening proportionately along a consistent flow–function relationship (Hotelling’s multivariate t-test for two variables).
The present study is the first to demonstrate a sizeable infarct size reduction following prolonged myocardial ischaemia/reperfusion with ivabradine, and this effect was partially independent of heart rate reduction.

**Conceptual framework of heart rate and regional myocardial ischaemia: supply vs. demand**

**Reversible ischaemic injury**

Our model of regional coronary hypoperfusion entails reversible ischaemic injury for the first 20 min of ischaemia, i.e. the timeframe in which data for the generation of a flow–function relationship were obtained. In this scenario, regional blood flow and contractile function are reduced proportionately along a consistent more or less linear flow–function relationship. Such relationship is shifted right-and downwards at increased heart rate. However, when blood flow is normalized for heart rate, i.e. calculated for each single cardiac cycle, there is a consistent relationship independent from heart rate. Such consistent flow–function relationship in regional myocardial ischaemia has led Ross to propose the concept of perfusion–contraction matching, i.e. there is no imbalance between regional supply, as determined by blood flow, and regional demand, as determined by contractile function, but rather a preserved balance which may act to adapt the myocardium even to more prolonged myocardial ischaemia. The data with ivabradine in the present study fully support this concept, in that ivabradine did not alter the balance between blood flow (supply) and contractile function (demand) but improved both flow and function proportionately along a consistent flow–function relationship when heart rate was reduced; no residual effect of ivabradine was seen when heart rate reduction was eliminated by atrial pacing. As expected, the data point with ivabradine and reduced heart rate is somewhat leftwards of the relationship of function to blood flow per minute (Figure 2A) and fully shifted onto the relationship of function to blood flow per beat (Figure 2B).

Thus, attenuation of reversible ischaemic injury by selective heart rate reduction with ivabradine in the present study is fully accredited to improved blood flow (supply) which then even permitted enhanced contractile function (demand) (Figure 2).

**Irreversible ischaemic injury**

Heart rate is an established determinant of infarct size. In the present study, when infarct size is plotted as a function of residual blood flow, the relationship with ivabradine is shifted downwards from that with placebo, indicating that infarct size is reduced at any given blood flow and suggesting that this benefit is secondary to reduced demand. In contrast, the cumulative work index during ischaemia is, if anything, increased with ivabradine, and this consideration would not favour the view that reduced infarct size is secondary to reduced demand. The issue is further confounded by the temporal and spatial development of infarction during myocardial ischaemia and reperfusion, since for methodological reasons infarct size can only be validly determined after sustained ischaemia and several hours reperfusion.

Part of the protection by ivabradine was independent of heart rate reduction and not eliminated by atrial pacing. This heart rate-independent protection can neither be attributed to improved
Table 3  Haemodynamics, contractile function, and regional myocardial blood flow of groups 7, 8, and 9

<table>
<thead>
<tr>
<th>Time</th>
<th>HR (1/min)</th>
<th>LVPmax (mmHg)</th>
<th>dP/dtmax (mmHg/s)</th>
<th>CAPmean (mmHg)</th>
<th>CBFmean (mL/min)</th>
<th>WTpost (%)</th>
<th>WTant (%)</th>
<th>LOOPaw (mmHg mm)</th>
<th>RMBF (mL/min/g)</th>
<th>RMBF (μL/beat/g)</th>
</tr>
</thead>
</table>
| Group 7  
(n=7) | Baseline  
95 ± 3 | 95 ± 3 | 1393 ± 56 | 123 ± 4 | 21.5 ± 2.1 | 19.2 ± 3.4 | 37.5 ± 4.1 | 278 ± 24 | 0.569 ± 0.035 | 5.974 ± 0.228 |
|        | IschS  
95 ± 3 | 79 ± 2* | 1023 ± 48* | 25 ± 3* | 3.5 ± 0.8* | 17.5 ± 4.4 | 2.2 ± 2.4* | 6 ± 5 | 0.071 ± 0.013* | 0.737 ± 0.110* |
|        | Isch30  
90 ± 1 | 83 ± 3* | 1070 ± 64* | 32 ± 7* | 3.4 ± 0.8* | 19.6 ± 4.8 | 2.0 ± 2.3* | 8 ± 5* | 1.129 ± 0.198* | 13.98 ± 3.740* |
|        | Isch60  
91 ± 2 | 84 ± 3* | 1178 ± 72* | 32 ± 8* | 3.4 ± 0.8* | 19.9 ± 4.9 | 1.3 ± 1.9* | 5 ± 6* | 0.532 ± 0.049 | 5.019 ± 0.931 |
|        | Isch90  
69 ± 3* | 78 ± 3* | 1056 ± 67* | 33 ± 8* | 3.5 ± 0.8* | 21.2 ± 5.7 | 2.1 ± 3.0* | 8 ± 8* | 0.073 ± 0.011* | 0.815 ± 0.138* |
|        | Rep10  
70 ± 4* | 75 ± 4* | 998 ± 129* | 121 ± 6 | 46.3 ± 4.7* | 20.4 ± 5.8 | 2.3 ± 1.8* | 7 ± 4* | 1.041 ± 0.171* | 10.58 ± 1.937 |
| Group 8  
(n=7) | Baseline  
94 ± 5 | 94 ± 2 | 1344 ± 72 | 114 ± 4 | 27.3 ± 2.7 | 17.7 ± 1.9 | 39.2 ± 5.5 | 284 ± 38 | 0.569 ± 0.035 | 5.974 ± 0.228 |
|        | IschS  
94 ± 6 | 80 ± 1* | 1025 ± 52* | 24 ± 1* | 3.8 ± 0.7* | 21.1 ± 2.9 | 2.7 ± 2.7* | 13 ± 12* | 0.071 ± 0.013* | 0.737 ± 0.110* |
|        | Isch30  
94 ± 7 | 85 ± 3* | 1100 ± 94* | 26 ± 2* | 3.8 ± 0.7* | 21.6 ± 3.1 | 3.0 ± 2.8* | 15 ± 13* | 1.129 ± 0.198* | 13.98 ± 3.740* |
|        | Isch60  
92 ± 6 | 86 ± 1* | 1232 ± 60 | 25 ± 1* | 3.8 ± 0.7* | 22.4 ± 3.2 | 4.3 ± 2.4* | 19 ± 12* | 0.532 ± 0.049 | 5.019 ± 0.931 |
|        | Isch90  
93 ± 6 | 83 ± 3* | 1148 ± 83 | 23 ± 1* | 3.8 ± 0.7* | 21.2 ± 4.6 | 3.9 ± 2.7* | 16 ± 12* | 0.073 ± 0.011* | 0.815 ± 0.138* |
|        | Rep10  
92 ± 6 | 75 ± 3* | 1066 ± 86* | 114 ± 9 | 54.7 ± 7.0* | 22.0 ± 4.0 | 4.3 ± 1.8* | 18 ± 10* | 1.052 ± 0.134* | 11.78 ± 1.875* |
| Group 9  
(n=8) | Baseline  
101 ± 3 | 99 ± 4 | 1527 ± 42 | 127 ± 3 | 23.3 ± 1.8 | 15.8 ± 2.8 | 40.7 ± 4.2 | 327 ± 25 | 0.619 ± 0.025 | 6.150 ± 0.272 |
|        | IschS  
100 ± 2 | 84 ± 4* | 1111 ± 57* | 28 ± 2* | 3.4 ± 0.5* | 16.1 ± 3.1 | -2.4 ± 0.9* | -1 ± 5* | 0.076 ± 0.013* | 0.765 ± 0.137* |
|        | Isch30  
100 ± 3 | 86 ± 3* | 1125 ± 55* | 32 ± 1* | 3.4 ± 0.5* | 15.6 ± 2.9 | -2.3 ± 0.9* | -2 ± 5* | 1.041 ± 0.171* | 10.58 ± 1.937 |
|        | Isch60  
100 ± 4 | 88 ± 4* | 1266 ± 60* | 29 ± 1* | 3.4 ± 0.5* | 13.8 ± 2.1 | -0.4 ± 0.7* | 5 ± 4* | 1.041 ± 0.171* | 10.58 ± 1.937 |
|        | Isch90  
101 ± 3 | 85 ± 5* | 1137 ± 44* | 30 ± 1* | 3.4 ± 0.5* | 9.9 ± 1.9 | -0.8 ± 0.9* | 4 ± 5* | 1.041 ± 0.171* | 10.58 ± 1.937 |
|        | Rep10  
102 ± 3 | 82 ± 5* | 1191 ± 67* | 108 ± 3* | 42.0 ± 5.9* | 12.1 ± 1.5 | 1.0 ± 0.9* | 15 ± 5* | 1.041 ± 0.171* | 10.58 ± 1.937 |

*P < 0.05 vs. baseline (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).

**P < 0.05 vs. Group 8 (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).

***P < 0.05 vs. Group 9 (two-way ANOVA for repeated measures and Fisher’s LSD post hoc tests).

Isch5/30/60/90: 5/30/60/90 min after the onset of ischaemia; rep10: 10 min after onset of reperfusion.

HR, heart rate; LVPmax, maximal left ventricular pressure; dP/dtmax, maximal rate of rise of left ventricular pressure; CAPmean, mean coronary perfusion pressure; CBFmean, mean coronary blood flow; WTpost, systolic wall thickening in the posterior wall; WTant, systolic wall thickening in the anterior wall; LOOPaw, instantaneous left ventricular pressure development–wall thickening product during systole in the anterior wall; RMBF, transmural blood flow in the area at risk either expressed per time or per beat.

Post hoc (Tukey’s HSD) tests were applied to detect differences between groups, and significant differences were considered at the 0.05 level.
supply nor reduced demand during ischaemia. Also, the protective effect of ivabradine when given just before reperfusion was heart rate-independent.

It is currently unclear whether such heart rate-independent effect relates to the presence of I_f-channels in LV myocardium, but the fact that ivabradine is still protective when given before reperfusion points towards mechanisms involved in attenuation of reperfusion injury and post conditioning.

Benefits of ivabradine extend also beyond the immediate infarction phase, and ivabradine attenuates fibrosis and remodelling and increases vascularity and function. At equal heart rate reduction, both metoprolol and ivabradine attenuated post-myocardial infarction remodelling in rats, but metoprolol also attenuated LV dilation and hypertrophy and affected subcellular calcium handling differently from ivabradine. The comparison of β-blockade to ivabradine in patients with coronary artery disease and LV dysfunction will be apparent from the BEAUTIFUL trial.

Conflict of interest: G.H. and R.S. have received honoraria for lectures, and G.H. has served as consultant to Servier. The authors A.S., P.G., P.v.C., and D.S. have no conflict of interest to declare.

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Figure 4 Infarct size (IS, in percent of area at risk, AAR) as a function of regional blood flow. Ivabradine, when given 5 min before reperfusion, shifts the relationship downwards (ANCOVA), reflecting reduced infarct size at any given blood flow. With ivabradine and atrial pacing, the regression line remains displaced downwards.

References
Cardioprotection by ivabradine

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