Calcitonin gene-related peptide in myocardial ischaemia and reperfusion in the pig

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Abstract

Objective: In myocardial ischaemia, slow conducting capsaicin-sensitive C-fibres are activated. Apart from the mediation of pain, activation of these fibres causes release of various peptides, such as calcitonin gene-related peptide (CGRP), which is a potent vasodilator. The aim of this study was to investigate the role of CGRP in the context of myocardial ischaemia in vivo. Methods: The left anterior descending coronary artery (LAD) was occluded during 45 min in 27 anaesthetised open-chest pigs. LAD flow, mean arterial pressure (MAP), heart rate, peak dP/dt, arterial and coronary venous concentration of CGRP was measured prior to ischaemia, and during 4 h of reperfusion. The extent of myocardial infarction was measured using staining with triphenyl tetrazolium chloride. Results: Retroinfusion of CGRP 100 µg into the ischaemic myocardium was associated with a more pronounced hyperaemia, and systemic hypotension, during early reperfusion. The infarct size in relation to the area at risk was not affected by CGRP or the CGRP antagonist CGRP 8–37, and averaged 67 ± 3%. There were no changes in plasma CGRP levels during ischaemia or reperfusion. Conclusion: Exogenously administered CGRP can cause systemic hypotension and augments postischaemic coronary flow. In this model, no cardioprotective effect of CGRP could be proven. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: C fibre; CGRP; CGRP 8–37; Ischemia; Pig; Reperfusion

1. Introduction

Myocardial ischaemia is usually associated with the sensation of pain [1]. Visceral pain is mediated by slow conducting C-fibres. A proportion of these fibres is characterised by their sensitivity to capsaicin, the pungent agent in certain hot peppers [2,3]. Upon activation these fibres release a variety of peptides, including calcitonin gene-related peptide (CGRP), which has potent cardiostimulatory and vasodilator properties [4].

Immunohistochemical studies have shown that CGRP is present in cardiac afferents, with high density around the coronary arteries including the left anterior descending artery (LAD) [5]. CGRP is released in the isolated guinea pig heart following activation of capsaicin-sensitive afferents by ischaemia and during low pH perfusion, which is well known to correlate with ischaemia-induced metabolic disturbances [4,6,7]. Release of CGRP by low pH has also been shown in other organs, such as the pig urinary bladder, [8] and rat stomach [9]. Furthermore, in patients with acute myocardial infarction, an almost two-fold increase in plasma CGRP within 24 h of hospital admittance has been observed [10].

Interestingly, acute administration of capsaicin has been demonstrated to decrease postischaemic impairment of cardiac function in isolated guinea pig and rat hearts [11]. Moreover, CGRP has a cardioprotective effect in postischaemic rat hearts, while the CGRP-receptor antagonist CGRP(8–37) impairs postischaemic cardiac function [12]. In the pig in vivo CGRP, but not substance P which is co-stored and -released together with CGRP, mimics capsaicin-induced coronary vasodilation [13]. In the present study, we have investigated the effects of CGRP and CGRP(8–37) in experimental myocardial ischaemia and reperfusion in the pig in vivo.
2. Methods

This study conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985).

2.1. Animal preparation

Twenty-seven farm pigs (25–36 kg) of either sex were premedicated with intramuscular ketamine hydrochloride (25 mg · kg\(^{-1}\); Parke-Davis, Morris Plains, NJ, USA) and atropine sulphate (0.04 mg · kg\(^{-1}\); NM Pharma, Stockholm, Sweden). Anaesthesia was induced by a bolus of intravenous sodium pentobarbitone (12–18 mg · kg\(^{-1}\); ACO, Umeå, Sweden) and maintained by a continuous infusion (6–10 mg · kg\(^{-1}\) · h\(^{-1}\)) of sodium pentobarbionate. The animals were intubated and ventilated with air and oxygen. Respiratory rate and tidal volume were adjusted to keep arterial blood pH, PO\(_2\) and PCO\(_2\) within the physiological range. Body temperature was kept at 38.0–39.0°C by means of a heating pad. A 7F catheter was placed in the superior vena cava for fluid administration. A 6F catheter was advanced from the femoral artery into the descending aorta for sampling of blood and for measurement of mean arterial blood pressure (MAP) via a Statham P23Db transducer (Statham, Hato Rey, Puerto Rico). A 7.5F microtip catheter transducer (Millar Instruments, Houston, TX, USA) was introduced through the carotid artery and advanced into the left ventricle (LV) for recording of the LV pressure and its first derivative (dP/dt). This is an indicator of global LV function, and local variations in contractility can not be detected by this technique. All variables were continuously recorded on a Grass 7B polygraph recorder (Grass Instruments, Quincy, MA, USA).

The heart was exposed through a sternotomy. A vessel loop was passed around the left anterior descending artery (LAD), at a position from which approximately the distal third of the artery would be occluded by tightening the snare. An ultrasonic flow probe model 2SB (Transonic Systems, NY, USA) was placed around the artery just proximal to the snare and connected to a Transonic 208 blood flow meter. A 4F double-lumen balloon-tipped catheter (Wygon, Aachen, Germany) was introduced through an 8F guiding catheter into the right external jugular vein and advanced into the anterior interventricular vein, which runs close and parallel to the LAD. The tip of the catheter was positioned at the intended level of arterial occlusion. The central lumen of the catheter was used for blood sampling from, or infusion of drugs into the myocardium supplied by the occluded portion of the LAD (for details see [14,15]). Before insertion of the balloon catheter heparin sodium (7500 IU) was given intravenously.

In four separate closed-chest pigs (30–32 kg) the dose–response relationship for CGRP in terms of systemic vasoreactivity, and the blocking properties of CGRP(8–37) were evaluated. A quadruple-lumen balloon-tipped catheter (Swan–Ganz, Baxter 93A-931H-7.5F, Baxter Healthcare, Irvine, USA) was introduced through the right external jugular vein and the tip of the catheter was positioned in the pulmonary artery. Central venous pressure (CVP) was monitored continuously via the Swan–Ganz catheter, and kept between 3 and 8 mmHg. Cardiac output (CO) was determined by thermodilution and calculated by a CO computer (COM-2, Baxter, USA). The average of three CO determinations within 10% of one another was used. Systemic vascular resistance (SVR) was then calculated as (MAP-CVP)/CO.

2.2. Study protocol

After 30 min stabilisation following instrumentation, the LAD was occluded for 45 min. Arterial blood samples and samples from the anterior interventricular vein were collected 5 min before ischaemia, at 25 min of ischaemia and at 5, 15, 30, 60,120, 180 and 240 min of reperfusion. Every hour 2500 IU of heparin sodium was given intravenously. Four groups of pigs received retroinfusion into the anterior interventricular vein of either 0.9% NaCl (vehicle, n = 6), 100 μg CGRP (CGRP 100, n = 5), 10 μg CGRP (CGRP 10, n = 6), or 2 mg CGRP(8–37) (CGRP(8–37) retro, n = 5). The infusion was given at a rate of 2 ml · min\(^{-1}\) during the last 15 min of ischaemia. The balloon just proximal to the tip of the 4F catheter was temporarily inflated to allow selective retroinfusion into, or sampling from the ischaemic/reperfused area. A fifth group of pigs (CGRP(8–37) i.v., n = 5) received 2 mg of CGRP(8–37) as an intravenous infusion over 5 min, started 15 min before ischaemia. In this group, the balloon was inflated during the last 15 min of ischaemia and during sampling as in the other groups. The preischaemic haemodynamic values in this group were recorded prior to i.v. infusion of the drug. In case of ventricular fibrillation, a 12-J DC shock was given, followed by repeated shocks with 30 J as needed.

In the dose–response experiments three consecutive doses of CGRP (1, 10 and 100 μg, respectively) were administered as a bolus in 5 ml saline via the central venous catheter. MAP, CVP and CO was recorded prior to and after each dose of CGRP. The haemodynamic variables were allowed to return to baseline levels between each CGRP dose. After 90 min stabilisation, the protocol was repeated during administration of 2 mg CGRP (8–37) as an i.v. infusion (30 ml) over 15 min.

2.3. Evaluation of myocardial ischaemia and infarct size

After 4 hours of reperfusion, the LAD was reoccluded at the original site, the ascending aorta was clamped and 1 ml · kg\(^{-1}\) of 2% Evan’s blue was injected into the coronary circulation to outline the ischaemic myocardium. The heart was then stopped by an injection of potassium chloride into the coronary circulation After extirpation and
rinsing of the heart, the right ventricle and the atria were removed. The LV was then cut in approximately 1-cm-thick slices from apex to base. The area of non-stained myocardium (area at risk) on the basal surface of each slice was outlined. The slices were then incubated for 10 min at 37°C in 0.8% triphenyl tetrazolium chloride which stains viable myocardium red [16,17]. The areas of necrosis and area at risk were determined by planimetry, and the extent of necrosis was expressed as percent of the area at risk.

### 2.4. CGRP assay

Blood samples were collected in tubes containing EDTA, and kept on ice until the end of the experiment. Samples were centrifuged at 1200 g for 10 min at 4°C and 1 ml plasma was separated and stored at −18°C until analysis. After ethanol extraction, lyophilization and redisolution in phosphate buffer CGRP was measured with a radioimmunoassay (RIA) kit from Peninsula Laboratories, Belmont, CA, USA.

### 2.5. Drugs

Human CGRPα and CGRP(8–37) were obtained from Peninsula (Belmont, CA, USA). The drugs were dissolved in 0.9% NaCl immediately before use.

### Table 1

Mean heart rate (HR), arterial pressure (MAP), left anterior descending coronary artery (LAD) flow and left ventricular peak \( dP/dt \) from preischaemia through 240 min of reperfusion in all five groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Preischaemia (25 min)</th>
<th>Ischaemia</th>
<th>Reperfusion (min)</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
</tr>
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<tr>
<td>Vehicle</td>
<td>10 (9)</td>
<td>125 (6)</td>
<td>132 (6)</td>
<td>122 (7)</td>
<td>122 (6)</td>
<td>123 (7)</td>
<td>128 (10)</td>
<td>123 (13)</td>
<td>128 (15)</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>78 (4)</td>
<td>74 (5)</td>
<td>74 (3)</td>
<td>77 (5)</td>
<td>76 (4)</td>
<td>77 (3)</td>
<td>78 (2)</td>
<td>73 (3)</td>
<td>73 (4)</td>
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</tr>
<tr>
<td>LAD flow</td>
<td>9.9 (1.0)</td>
<td>0 (0)</td>
<td>25.6 (6.3)</td>
<td>26.4 (1.3)</td>
<td>24.8 (3.0)</td>
<td>22.7 (2.9)</td>
<td>18.7 (2.6)</td>
<td>12.1 (1.6)</td>
<td>8.0 (0.8)</td>
<td></td>
</tr>
<tr>
<td>( dP/dt )</td>
<td>1983 (266)</td>
<td>1650 (209)</td>
<td>1633 (133)</td>
<td>1717 (164)</td>
<td>1600 (124)</td>
<td>1650 (223)</td>
<td>1533 (223)</td>
<td>1517 (300)</td>
<td>1717 (369)</td>
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<tr>
<td>CGRP 100 μg</td>
<td></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>HR</td>
<td>129 (10)</td>
<td>137 (14)</td>
<td>160 (13)</td>
<td>156 (11)</td>
<td>153 (12)</td>
<td>162 (13)</td>
<td>150 (12)</td>
<td>153 (8)</td>
<td>156 (12)</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>85 (4)</td>
<td>85 (5)</td>
<td>55 (3)</td>
<td>64 (2)</td>
<td>71 (2)</td>
<td>67 (5)</td>
<td>71 (2)</td>
<td>74 (3)</td>
<td>72 (4)</td>
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<tr>
<td>LAD flow</td>
<td>12.7 (1.4)</td>
<td>0 (0)</td>
<td>21.6 (2.4)</td>
<td>32.4 (5.1)</td>
<td>45.0 (5.4)</td>
<td>36.8 (3.3)</td>
<td>29.2 (1.9)</td>
<td>20.6 (4.2)</td>
<td>12.1 (2.4)</td>
<td></td>
</tr>
<tr>
<td>( dP/dt )</td>
<td>1840 (238)</td>
<td>1560 (196)</td>
<td>2080 (265)</td>
<td>1860 (125)</td>
<td>1960 (183)</td>
<td>1760 (194)</td>
<td>1440 (103)</td>
<td>1660 (306)</td>
<td>1760 (343)</td>
<td></td>
</tr>
<tr>
<td>CGRP 10 μg</td>
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<td></td>
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<tr>
<td>HR</td>
<td>138 (7)</td>
<td>128 (15)</td>
<td>122 (19)</td>
<td>133 (7)</td>
<td>133 (5)</td>
<td>138 (7)</td>
<td>145 (5)</td>
<td>154 (6)</td>
<td>157 (7)</td>
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<tr>
<td>MAP</td>
<td>93 (6)</td>
<td>86 (8)</td>
<td>86 (7)</td>
<td>87 (5)</td>
<td>93 (5)</td>
<td>94 (5)</td>
<td>96 (7)</td>
<td>96 (9)</td>
<td>94 (9)</td>
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<tr>
<td>LAD flow</td>
<td>13.9 (1.3)</td>
<td>0 (0)</td>
<td>28.8 (2.9)</td>
<td>28.3 (4.4)</td>
<td>36.4 (3.9)</td>
<td>34.5 (3.3)</td>
<td>27.2 (3.0)</td>
<td>20.1 (3.6)</td>
<td>11.9 (1.9)</td>
<td></td>
</tr>
<tr>
<td>( dP/dt )</td>
<td>2760 (273)</td>
<td>2280 (258)</td>
<td>2280 (267)</td>
<td>2360 (234)</td>
<td>2380 (169)</td>
<td>2100 (224)</td>
<td>2100 (228)</td>
<td>2080 (246)</td>
<td>2000 (281)</td>
<td></td>
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<tr>
<td>CGRP(8–37) retro</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>HR</td>
<td>139 (17)</td>
<td>137 (14)</td>
<td>139 (8)</td>
<td>134 (10)</td>
<td>135 (10)</td>
<td>142 (11)</td>
<td>152 (14)</td>
<td>149 (15)</td>
<td>156 (14)</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>74 (4)</td>
<td>75 (6)</td>
<td>80 (7)</td>
<td>79 (7)</td>
<td>79 (8)</td>
<td>78 (6)</td>
<td>81 (5)</td>
<td>75 (7)</td>
<td>65 (9)</td>
<td></td>
</tr>
<tr>
<td>LAD flow</td>
<td>10.3 (1.4)</td>
<td>0 (0)</td>
<td>26.0 (3.2)</td>
<td>28.2 (3.5)</td>
<td>28.1 (3.0)</td>
<td>27.6 (2.2)</td>
<td>23.8 (2.7)</td>
<td>14.6 (2.5)</td>
<td>9.5 (1.4)</td>
<td></td>
</tr>
<tr>
<td>( dP/dt )</td>
<td>1720 (294)</td>
<td>1750 (386)</td>
<td>1860 (204)</td>
<td>1760 (232)</td>
<td>1760 (232)</td>
<td>2140 (469)</td>
<td>2440 (670)</td>
<td>2160 (403)</td>
<td>2080 (306)</td>
<td></td>
</tr>
<tr>
<td>CGRP(8–37) i.v.</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HR</td>
<td>128 (6)</td>
<td>132 (8)</td>
<td>141 (11)</td>
<td>144 (13)</td>
<td>148 (15)</td>
<td>153 (13)</td>
<td>157 (10)</td>
<td>152 (9)</td>
<td>157 (9)</td>
<td></td>
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<tr>
<td>MAP</td>
<td>91 (2)</td>
<td>93 (3)</td>
<td>93 (4)</td>
<td>92 (4)</td>
<td>88 (2)</td>
<td>87 (3)</td>
<td>80 (10)</td>
<td>77 (7)</td>
<td>71 (6)</td>
<td></td>
</tr>
<tr>
<td>LAD flow</td>
<td>15.7 (2.0)</td>
<td>0 (0)</td>
<td>36.8 (5.7)</td>
<td>40.4 (6.7)</td>
<td>40 (3.7)</td>
<td>36.8 (3.5)</td>
<td>32.5 (4.2)</td>
<td>24.4 (3.9)</td>
<td>15.7 (3.5)</td>
<td></td>
</tr>
<tr>
<td>( dP/dt )</td>
<td>2340 (87)</td>
<td>2700 (283)</td>
<td>2760 (331)</td>
<td>3080 (422)</td>
<td>3080 (422)</td>
<td>2900 (303)</td>
<td>2440 (350)</td>
<td>2420 (191)</td>
<td>2200 (261)</td>
<td></td>
</tr>
</tbody>
</table>

In the CGRP(8–37) i.v. group, the shown preischaemic values were recorded prior to infusion of the drug. Values are presented as means (s.e.m.).

### 2.6. Statistical evaluation

Since the experimental protocol involved collection of data over time in five different groups, the absolute data were analysed according to a repeated measures analysis of variance (ANOVA), with time (from preischaemia values through 240 min of reperfusion) as the repeated measures factor, and group (vehicle, CGRP 100, CGRP 10, CGRP(8–37) retro, and CGRP(8–37) i.v.) as the between-groups factor. When appropriate, Scheffe’s multiple comparison procedure among means was used. If the interaction between group and time was significant, simple main effects were examined. The \( P \)-values were then corrected according to the Bonferroni procedures. Particular measures were constructed from the repeated observations within each pig, such as the difference between preischaemic values and values after 240 min of reperfusion, and means over selected time points. These new variables were then analysed by a one-way ANOVA.

The effects during ischaemia were analysed separately with a repeated measures ANOVA (preischaemia and ischaemia). Infarct size and number of DC shocks were analysed with one-way ANOVA for independent samples. Paired and unpaired \( t \)-test was used in the dose–response study. Differences were considered significant at \( P < 0.05 \). All values are given as mean ± s.e.m.
3. Results

The haemodynamic data are shown in Table 1. In the CGRP(8–37) i.v. group (where the drug was administered prior to ischaemia) haemodynamic data were also recorded 10 min after the infusion, to evaluate a possible effect of the drug on basal haemodynamics. There was no such effect.

3.1. Dose–response study

Baseline MAP and SVR were 113 ± 3 mmHg and 26 ± 1 mmHg min/l, respectively. CGRP 1, 10 and 100 μg caused reduction of MAP to 100 ± 1% (n.s.), 89 ± 2% \(P < 0.05\), and 55 ± 3% \(P < 0.001\) of the level before CGRP bolus, respectively (Fig. 1a). SVR was reduced to 97 ± 5% (n.s.), 93 ± 5% (n.s.) and 63 ± 7% \(P < 0.05\) of the level before CGRP bolus, respectively (Fig. 1b).

CGRP(8–37) did not influence baseline haemodynamic variables. The response to CGRP was attenuated at all three doses, with statistical significance for MAP at the 100-μg dose MAP 66 ± 4% vs. 55 ± 3%, \(P < 0.05\) (Fig. 1a,b).

3.2. LAD flow

For all five groups, there was a significant \(P < 0.001\) change in LAD flow over time, with peak values occurring at 15–30 min of reperfusion (Fig. 2). There was a difference \(P = 0.057\) in the time course between the groups, constituted by a more marked peak in the CGRP 100 group. After 240 min of reperfusion, the LAD flow had returned to preischaemia levels in all five groups.

3.3. Mean arterial pressure

There was a significant \(P < 0.001\) difference in the time course between the five groups (Fig. 3). This interaction was accounted for by the very marked changes over time \(P < 0.001\) in the CGRP 100 group with a drop at 5 min of reperfusion to 55 ± 3 vs. 74 ± 3 mmHg in the vehicle group \(P < 0.05\). There was no change in MAP from the preischaemia value to the value observed after 25 min of ischaemia (84 ± 2 vs. 82 ± 3 mmHg).
3.4. Peak $dP/dt$

Overall ($n = 27$), there was no significant change over time in $dP/dt$ (Fig. 4). There was no change in $dP/dt$ from the preischaemia value to the value observed after 25 min of ischaemia ($2123 \pm 205$ vs. $1984 \pm 203$ mmHg/s).

3.5. Heart rate

There was an overall increase in heart rate ($P < 0.01$) in the five groups together ($n = 27$) over time, but no significant difference in time course between the groups (Fig. 5). Thus, the mean heart rate after 240 min of reperfusion ($150 \pm 5$) was significantly higher ($P < 0.01$) compared to mean heart rate before ischaemia ($133 \pm 4$). There was no change in heart rate from the preischaemia value to the value observed after 25 min of ischaemia.

3.6. DC shocks

Three animals in the CGRP 100 group, one in the CGRP 10 group, one in the CGRP(8–37) retro group and two in the vehicle group had no ventricular fibrillation. The remaining 20 animals had one or more episodes of ventricular fibrillation, usually commencing after 20–25 min of ischaemia, and received up to ten DC shocks.

3.7. Infarct size

In the five groups overall, the size of the myocardial area at risk expressed as percent of the LV was $21 \pm 1\%$ ($n = 27$), and did not differ between the groups. The infarct size in relation to the area at risk was $67 \pm 3\%$ ($n = 27$), without any difference between the groups (Fig. 6).

3.8. CGRP levels

In the vehicle group, preischaemic arterial and coronary venous plasma levels of CGRP were $11 \pm 1$ and $16 \pm 2$ pmol/L, respectively. This difference was not significant, and the levels did not change during ischaemia or reperfusion.

4. Discussion

4.1. Main findings

In the present study, retrograde infusion of 100, but not 10 $\mu$g CGRP was associated with a more pronounced postischaemic hyperaemia during early reperfusion. Retroinfusion of 100 $\mu$g CGRP was also associated with a drop in systemic blood pressure during early reperfusion. Neither retroinfusion of CGRP, nor CGRP(8–37) by retro- or intravenous infusion had any effect on infarct size. Furthermore, i.v. CGRP evoked a dose-dependent decrease in MAP and SVR, partly attenuated by CGRP(8–37).
4.2. Administration and dose of CGRP and CGRP(8–37)

Porcine coronary collateral flow in the range 1–2% of non-ischaemic flow has been reported by several groups [18–20]. Coronary venous retroinfusion of various drugs in coronary ligated experimental animals induces substantially higher concentrations of the drug in the ischaemic myocardium than does i.v. administration 14. In the same pig model as the one we have used, Ryden et al. have shown 4 (subendocardial) to 154 (subepicardial) times higher drug concentration in the ischaemic compared to the non-ischaemic myocardium, using retroinfusion. In the non-ischaemic border zone, the drug concentration was only 1.4–1.6 times that in non-ischaemic myocardium [14]. Thus, in the pig, retroinfusion efficiently delivers the drug to the ischaemic myocardium without significant influence from collateral flow.

In pigs and dogs, intracoronary administration of CGRP in doses ranging from 2.5 to 250 pmol/kg resulted in dose-related increase in coronary diameter and/or flow [13,21,22]. Based on these data, and on the systemic dose–response relationships presented in this paper, we have used 10 and 100 μg of CGRP, corresponding to approximately 100 and 1000 pmol/kg, the objective being to use the highest dose that would not cause deleterious systemic hypotension. In vitro experiments on pig coronary arteries, and in vivo experiments in dogs, have shown that administration of the CGRP antagonist CGRP(8–37) in concentrations 10–1000 times higher than the concentration of CGRP was effective in counteracting the vasodilating effect of exogenous CGRP [21,23]. We have presently used a dose of CGRP(8–37) that was approximately 25 and 250 times, respectively, higher than the investigated doses of CGRP. Our experiments with retroinfusion of CGRP(8–37) during ischaemia indicated that this had no effect on LAD flow and systemic haemodynamics. Recordings from vagal afferents during 40 min coronary occlusion have shown that increase in left ventricular receptor activity peaks already after 30 s of occlusion. A second increase in receptor activity occurs immediately after release of the occlusion [24]. We therefore added the group CGRP(8–37) i.v., the objective being to achieve receptor blockade before start of ischaemia. However, in our study, the administration of CGRP(8–37) caused only a minor attenuation of the systemic effects of exogenous CGRP. It therefore remains unclear as to what extent CGRP(8–37) blocks a possible effect of endogenous CGRP on postischaemic coronary hyperaemia.

4.3. Haemodynamic effects of CGRP and CGRP(8–37)

Retroinfusion of CGRP was associated with more pronounced post-ischaemic hyperaemia during early reperfusion. MAP is an important determinant of perfusion, and should be taken into account in the evaluation of changes in coronary flow. Thus, the augmented hyperaemia in the CGRP group occurred in spite of a very marked fall in MAP, indicating a more pronounced vasodilating effect in the coronary circulation than in the systemic circulation.

4.4. Infarct size

This study showed no effect of CGRP or CGRP(8–37) on infarct size. Interestingly, the coronary flow was not lower after 240 min of reperfusion compared to preischaemia flow in any of the groups, including the vehicle group. Thus, the no-reflow phenomenon, which has been suggested to play an important role in the development of reperfusion injury did not occur in our study [25–27]. Nevertheless, the infarct size (67 ± 3% of the area at risk) and the area at risk (21 ± 1% of the LV) were in the range reported by other groups in untreated control animals [27,28]. This extent of myocardial infarction, in spite of a preserved coronary flow after 240 min of reperfusion, indicates that the no-reflow phenomenon may not be that important in the causation of ischaemia/reperfusion injury. Indeed, other mechanisms than augmentation of coronary flow have been suggested to explain the cardioprotective properties of various drugs [29].

It has been proposed that endogenous myocardial protective substances play an important role in ischaemic preconditioning [12,30,31]. In the isolated rat heart, CGRP pretreatment resulted in improved postischaemic cardiac performance comparable to the improvement seen with ischaemic preconditioning [12,31]. This improvement was abolished by CGRP(8–37), but CGRP(8–37) alone had no effect on postischaemic recovery. In the present study, the CGRP-antagonist CGRP(8–37), administered either locally by retroinfusion, or systemically by intravenous infusion, did not influence the postischaemic cardiac function or infarct size, which may suggest that locally released CGRP does not function as a cardioprotective agent in this experimental model.

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