MYOCARDIAL OUTFLOW OF CALCITONIN GENE-RELATED PEPTIDE IN RELATION TO METABOLIC STRESS DURING CORONARY ARTERY BYPASS GRAFTING WITHOUT CARDIOPULMONARY BYPASS

Göran Källner, MD^a

Anders Öwall, MD, PhD^b

Anders Franco-Cereceda, MD, PhD^a

Objective: Because of adverse effects of cardiopulmonary bypass and the prospect of shortening intensive care and hospital stay, coronary artery bypass grafting without cardiopulmonary bypass is gaining increased attention. The impact of the localized myocardial ischemia that is inherent in these procedures has not been thoroughly investigated in human beings. We have investigated metabolic changes, possible myocardial damage, and myocardial outflow of the vasodilator calcitonin gene-related peptide during coronary artery bypass grafting without cardiopulmonary bypass. **Methods:** Coronary sinus and arterial blood was sampled before coronary arterial occlusion, after 10 minutes of ischemia, and after 1 and 10 minutes of reperfusion in 9 consecutive patients (mean age 70 ± 5 years) who had an anastomosis performed to the left anterior descending artery without cardiopulmonary bypass. **Results:** No perioperative myocardial infarctions occurred. The arteriovenous difference in lactate decreased during ischemia, to reach a minimum after 1 minute of reperfusion (-0.17 ± 0.25 vs 0.15 ± 0.25 mmol/L before ischemia; $P = .008$). Myocardial lactate extraction decreased (from 11.2 ± 13.6 μ mol/min before ischemia to -3.0 ± 7.0 μ mol/min after 1 minute of reperfusion; $P = .012$), that is, a net production of lactate. The arteriovenous difference in calcitonin gene-related peptide decreased from -0.1 ± 2.6 pmol/L before ischemia to -30.5 ± 26.5 pmol/L ($P = .008$) after 1 minute of reperfusion. **Conclusions:** The localized myocardial ischemia associated with these procedures causes metabolic changes in the myocardium, but no myocardial damage. The ischemia-related outflow of calcitonin gene-related peptide indicates that the vasodilating and cardioprotective properties of this peptide that are known from animal studies may be of importance in myocardial ischemia in human beings. (J Thorac Cardiovasc Surg 1999;117:447-53)

Coronary artery bypass grafting (CABG) is routinely performed with cardiopulmonary bypass (CPB), with excellent results. However, CPB has been associated with several adverse effects,^{1,2} and CABG without

CPB has recently gained increased attention.³ The short-term to midterm clinical outcome in these operations in terms of morbidity and mortality has been characterized,^{4,5} and the results are encouraging. In these operations, coronary flow in the vessel being bypassed has to be interrupted, usually for 10 to 20 minutes, while the distal anastomosis is constructed. The myocardial metabolic stress that results from this intraoperative temporary interruption of coronary blood flow has not been thoroughly investigated in human beings. In experimental ischemia in the pig, a 300% increase in tissue lactate was observed within 15 minutes,⁶ and lactate production is widely used as an indicator of anaerobic metabolism.⁷ In clinical situations such as conventional CABG,⁸ unstable angina,⁹ and radiofrequency catheter ablation,¹⁰ troponin T has proved to be a highly sensitive and specific tool in evaluating even minor myocardial damage,

From the Departments of Thoracic Surgery^a and Cardiothoracic Anesthesia,^b Karolinska Hospital, Stockholm, Sweden.

Supported by grants from the Wallenberg Foundation, the Swedish Heart-Lung Foundation, the Swedish Medical Research Council, and funds from the Karolinska Institute.

Received for publication June 1, 1998; revisions requested Aug 19, 1998; revisions received Sept 8, 1998; accepted for publication Oct 7, 1998.

Address for reprints: Göran Källner, MD, Department of Cardiothoracic Surgery and Anesthesiology, Karolinska Institute at Huddinge University Hospital, S-141 86 Huddinge, Sweden.

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0022-5223/99 \$8.00 + 0 12/1/95022

Table I. Preoperative and intraoperative data

Patient No.	Sex	Age (y)	Previous MI	LV function	LAD occluded	Grafted vessels	LAD ischemia (min)	Pre-conditioning (min)	Incision
1	M	73	No MI	Mild	No	LAD + diag	19	0	Sternotomy
2	M	68	A + I	Mild	Yes	LAD	16	0	LAST + sternotomy
3	F	75	A	Normal	No	LAD + diag	14	3	Sternotomy
4	M	58	A	Moderate	Yes	LAD	9	2	Sternotomy
5	M	77	A + I	Mild	Yes	LAD + RCA	22	3	Sternotomy
6	M	73	A	Normal	No	LAD	17	3	LAST
7	M	73	A	Mild	No	LAD + diag	13	2	Sternotomy
8	M	65	A	Mild	No	LAD + RCA	19	2	Sternotomy
9	F	71	No MI	Normal	No	LAD + diag + RCA	16	3	Sternotomy

MI, Myocardial infarction (A, anterior; I, inferior); LV, left ventricular (mild, mildly depressed function; moderate, moderately depressed function); LAD, left anterior descending coronary artery; diag, diagonal branch; RCA, right coronary artery; LAST, left anterior small thoracotomy.

but information on release of troponin T in CABG performed without CPB is scarce.¹¹

In myocardial ischemia, slow-conducting C-fiber afferents are activated.^{12,13} This activation is associated with release of calcitonin gene-related peptide (CGRP), which has potent chronotropic and vasodilator effects.¹³ In experimental models of myocardial ischemia, C-fiber activation with CGRP release causes improvement of postischemic cardiac function.¹⁴ Furthermore, in patients with acute myocardial infarction, an almost 2-fold increase in plasma CGRP within 24 hours of hospital admittance has been observed.¹⁵ CABG without CPB represents a unique opportunity to study the effects of a well-defined and controlled ischemic episode in human beings in vivo. The aim of the present study was to evaluate myocardial metabolic disturbance and possible myocardial damage during CABG without CPB and to investigate to what extent such disturbance is associated with activation of cardiac C-fibers and outflow of C-fiber peptides.

Patients and methods

Patients. This study was approved by the Karolinska Hospital Human Research Committee. Patients requiring grafts in the left anterior descending coronary artery (LAD) system only or in the LAD system and right coronary artery system were offered surgery without CPB. Our present policy is to use CPB to perform circumflex grafts. Further exclusion criteria for surgery without CPB were significant left main stenosis and severely depressed left ventricular function. All patients who underwent elective coronary surgery without CPB from January 1998 through April 1998 were eligible for the study and were included after informed consent. Our study group consisted of 7 men and 2 women. All patients but 2 had had 1 or more myocardial infarctions. Three patients had an occluded LAD; the remaining 6 patients had significant LAD stenosis. Left ventricular func-

tion was classified as normal, mildly depressed, or moderately depressed as assessed from angiography or perioperative transesophageal echocardiography, or both studies. The preoperative and intraoperative data are shown in Table I.

Anesthetic and surgical technique. Intramuscular premedication with morphine and scopolamine was given 1 hour before anesthesia. Anesthesia was induced with thiopental, midazolam, and fentanyl and was maintained with a continuous infusion of midazolam and fentanyl. Pancuronium was given before endotracheal intubation, followed by normocapnic, volume-controlled ventilation with 50% oxygen in air. Heparin (1 mg/kg) was given before distal division of the left internal thoracic artery (LITA). The radial artery was cannulated for continuous blood pressure recording and blood sampling. The 5-lead electrocardiogram (ECG) with automatic analysis of the ST segment was continuously monitored, and all patients were monitored with transesophageal echocardiography. Esmolol was given as requested by the surgeon, starting with a loading dose of 0.5 mg/kg and then as an infusion at 50 to 300 µg/kg per minute.

The operation was performed through a midline sternotomy or through a left anterior small thoracotomy, according to the surgeon's preference. The LITA was harvested for anastomosis to the LAD. Additional grafts were performed with the saphenous vein or the left radial artery. When multiple vessels were bypassed, the anastomosis to the LAD was constructed first, except in 1 patient in whom a diagonal branch was anastomosed before the LAD for anatomic reasons. In this patient the preischemic samples were drawn before diagonal branch ischemia. A 2-0 Prolene suture (Ethicon, Inc, Somerville, NJ) was passed around the LAD just proximal and just distal to the anastomotic site. The snares were tightened for 2 to 3 minutes for ischemic preconditioning and to test the tolerability of the ischemia (patients 3-9). After 4 to 5 minutes of reperfusion the snares were tightened again to ensure an operative field as bloodless as possible. The anastomotic site was stabilized with an Origin OMS-CS cardiac stabilizer (Origin USA, Menlo Park, Calif). The anastomosis was then constructed with a continuous 8-0 Prolene suture.

After completion of the LITA-LAD anastomosis, the snares and the occluder on the LITA were released. Additional anastomoses were not started before at least 10 minutes had elapsed after re-establishment of LAD flow. Intraoperative data are shown in Table I.

Study protocol. A thermodilution catheter (Webster Laboratories, Altadena, Calif) was inserted into the coronary sinus through the right internal jugular vein under fluoroscopic guidance.¹⁶ The catheter was advanced to the proximal part of the great cardiac vein. The correct position in the coronary sinus was verified by analysis of the blood oxygen saturation. Coronary sinus blood flow was measured in duplicate by the retrograde thermodilution technique, with the use of saline solution.¹⁷ This catheter was used for coronary sinus blood sampling and flow measurements at the following times: after opening of the pericardium but before LAD ischemia, at 10 minutes of LAD ischemia, 1 minute after re-establishment of LAD flow, and 10 minutes after re-establishment of LAD flow. Simultaneous samples were drawn from the radial artery. Each sample was immediately analyzed for total oxygen content with the use of an OSM 3 Hemoximeter connected to an ABL 505 blood gas analyzer (Radiometer A/S, Copenhagen, Denmark). The samples were collected in vacuum tubes (containing sodium fluoride for the lactate analysis, ethylenediaminetetraacetic acid for analysis of CGRP, and gel barrier without anticoagulation for the analysis of troponin T), kept in ice slush, and centrifuged (10 minutes, 4°C) at 1620g within 30 minutes of collection of the last sample. The plasma was then immediately frozen at -70°C and stored until analysis. Heart rate, mean arterial pressure, and ST-segment deviation were recorded at each sampling point with a Tram 600 SL module, connected to a Tramscope 12C (Marquette Electronics Inc., Milwaukee, Wis). On postoperative day 1, a 12-lead ECG was recorded, and peripheral venous blood samples were drawn for routine analysis of creatine kinase, aspartate aminotransferase, and alanine aminotransferase concentrations. Our ECG criteria for transmural myocardial infarction were new Q waves of more than 0.03 second in duration in at least 2 anatomically adjacent leads. A localized ST-segment elevation followed by T-wave inversion in at least 2 adjacent leads was considered diagnostic of acute subendocardial myocardial infarction. In the absence of ECG changes, a non-Q wave infarction was diagnosed by means of the following enzymatic criteria (units per liter): aspartate aminotransferase more than 180 (with alanine aminotransferase <60 and creatine kinase >1200). The upper reference limits in our laboratory are 48 U/L for aspartate aminotransferase and alanine aminotransferase and 1200 U/L for creatine kinase.

Lactate in plasma was determined with the use of a "dry chemistry" kit from Vitros (Johnson & Johnson, New York, NY) and analyzed spectrophotometrically in a Vitros 950 analyzer. The range of the analysis is 0.50 to 12.00 mmol/L, and the reference interval is 0.7 to 2.1 mmol/L. Cardiac troponin T in serum was determined with the second-generation troponin T enzyme-linked immunosorbent assay (Enzymun-Test Troponin-T) on the ES 300 system (Boehringer Mannheim

GmbH, Mannheim, Germany). The lower detection limit is 0.01 µg/L, the cutoff value for myocardial damage is 0.1 µg/L, and the cross-reactivity with skeletal muscle troponin T is less than 0.01%.¹⁸ CGRP was analyzed with radioimmunoassay with the use of antibody raised against human CGRP-α (Peninsula Laboratories Inc, Belmont, Calif).¹⁹

Calculations and statistical evaluation. For oxygen, lactate, and CGRP, the concentration in coronary venous plasma was subtracted from the concentration in arterial plasma to form the arteriovenous concentration difference. The myocardial extraction (or outflow) of substances was calculated by multiplying the arteriovenous concentration difference by the flow in the coronary sinus, corrected for hematocrit value. These parameters and mean arterial pressure, heart rate, and coronary sinus blood flow were then evaluated by the Friedman analysis of variance by ranks test,²⁰ using STATISTICA software, version 5.1 (StatSoft Inc, Tulsa, Okla). If this indicated significance at the .05 level, a Wilcoxon matched pairs test between the peak values and the preischemic values was done. Values are expressed as mean ± SD.

Results

Clinical outcome. The in-hospital mortality was zero, and all patients were discharged from the hospital within 6 to 8 days. No perioperative myocardial infarctions occurred. In patient 2, a conversion from left anterior small thoracotomy to sternotomy was done because of technical difficulties with the LITA.

Signs of ischemia and metabolic adjustment. Mean arterial pressures before ischemia, at 10 minutes of ischemia, and after 10 minutes of reperfusion were 71 ± 11 , 67 ± 6 , and 65 ± 13 mm Hg, respectively. This slight decrease in blood pressure was not significant ($P = .32$). Likewise, heart rate did not change significantly over time (70 ± 13 , 71 ± 27 , and 67 ± 20 beats/min, respectively; $P = .067$). The automatic analysis of the ST segment showed no signs of ischemia in any of the patients; any aberrations were within 0.1 mV.

The preischemic blood flow measured in the coronary sinus was 64 ± 53 mL/min. There was a trend toward elevated flow at 1 and 10 minutes of reperfusion (Fig 1). Before myocardial ischemia the arterial and coronary sinus lactate levels were 1.11 ± 0.35 and 0.96 ± 0.26 mmol/L, respectively. Although the arterial lactate level did not change significantly ($P = .47$) during or after ischemia, a significant increase in coronary sinus lactate was noted at 1 minute of reperfusion (1.25 ± 0.25 mmol/L, $P = .015$). The arteriovenous difference in lactate concentration decreased during ischemia to reach a minimum after 1 minute of reperfusion (-0.17 ± 0.25 vs 0.15 ± 0.25 mmol/L before ischemia; $P = .008$). Myocardial lactate extraction also decreased (from 11.2 ± 13.6 µmol/min before ischemia to $-3.0 \pm$

Fig 1. Coronary sinus blood flow. *isch*, Ischemia; *rep*, reperfusion.

Fig 2. Arterial and coronary sinus lactate levels and arteriovenous difference in lactate level (*AV diff*) on the left Y-axis, and myocardial lactate extraction on the right Y-axis. * $P < .05$. ** $P < .01$ for lactate levels and *AV diff* versus preischemic values. +++ $P < .01$ for lactate extraction versus preischemic values.

7.0 $\mu\text{mol/min}$ after 1 minute of reperfusion; $P = .012$) (Fig 2), that is, a net production of lactate. The arteriovenous oxygen concentration difference tended to be lower at 1 minute of reperfusion compared with the preischemic level (82 ± 13.0 vs 95 ± 20 mL/L), whereas the myocardial oxygen extraction was elevated (7.2 ± 5.2 vs 6.1 ± 5.3 mL/min) (Fig 3). These changes were not significant ($P = .17$ and $P = .58$, respectively).

Patient 3 had a slight elevation in coronary sinus troponin T level ($0.35 \mu\text{g/L}$) in the preischemic sample. All other troponin T samples were within the normal range.

Outflow of CGRP. The preischemic arterial and coronary sinus plasma levels of CGRP were 5.1 ± 3.5 and 5.1 ± 3.9 pmol/L, respectively. The arterial level of CGRP showed a minimal variation ($P = .20$) during ischemia and reperfusion, whereas at 1 minute of reperfusion there

was a marked increase in CGRP in the coronary sinus (34.9 ± 25.7 vs 5.1 ± 3.9 pmol/L before ischemia; $P = .008$), paralleled by a corresponding decrease in arteriovenous difference in CGRP level (from -0.1 ± 2.6 pmol/L before ischemia to -30.5 ± 26.5 pmol/L after 1 minute of reperfusion; $P = .008$) (Fig 4). Myocardial outflow of CGRP increased (from 0.08 ± 0.27 pmol/min before ischemia to 1.84 ± 2.34 pmol/min after 1 minute of reperfusion; $P = .012$) (Fig 4).

Discussion

This study shows that the relatively short-lasting and limited ischemia that is associated with CABG without CPB induces adjustments in lactate metabolism. Furthermore, outflow of CGRP increased immediately on reperfusion of the ischemic myocardium, indicating C-fiber activation. There were, however, no hemody-

Fig 3. Total arterial and coronary sinus oxygen concentration (CaO_2 and CvO_2 , respectively), and arteriovenous oxygen difference ($AV O_2$ diff) on the left Y-axis, and oxygen consumption (Vo_2) on the right Y-axis.

Fig 4. Arterial and coronary sinus CGRP levels and arteriovenous difference in CGRP level (AV diff) on the left Y-axis and CGRP extraction on the right Y-axis. ** $P < .01$ for CGRP levels and AV diff versus preischemic values. ++ $P < .01$ for CGRP extraction versus preischemic values.

namic or ECG changes during the operative procedure. The heart normally extracts lactate as a part of aerobic metabolism.²¹ Although myocardial lactate production is considered to be a good indicator of myocardial ischemia,⁷ it should be kept in mind that coronary sinus lactate levels only vaguely reflect the substantially elevated lactate levels in ischemic tissue.⁶ Thus the present finding of myocardial lactate production during and early after ischemia most likely represents true myocardial ischemia. In this context, ST-segment changes in the ECG are of very limited value, particularly because of low sensitivity.⁷ The absence of ST-segment changes in the present study thus has little bearing on the assessment of ischemia.

Under basal conditions, the heart extracts a high and

relatively fixed percentage of the oxygen from the coronary arterial blood. Therefore the oxygen debt that results from an ischemic episode must be met mainly by an increased myocardial perfusion. In our study the myocardial arteriovenous oxygen concentration difference actually *decreased* during early reperfusion, while oxygen consumption *increased*. Although none of these changes were statistically significant, it might be assumed that in the course of the postischemic hyperemia (caused by metabolic, humoral, and neuronal mediators) some shunting of blood occurs in, and adjacent to, the previously ischemic myocardium.

The study group shows considerable heterogeneity in some important respects. Seven patients had had a previous anterior myocardial infarction, and 3 patients had

an occluded LAD. Thus the amount of viable myocardium exposed to ischemia during the procedure probably differed considerably among the patients. A larger proportion of patients with an open, stenosed LAD and a noninfarcted anterior wall would probably have yielded more obvious results in the studied parameters, including coronary sinus flow and oxygen consumption.

In accord with Hadjinikolaou and associates,¹¹ we did not detect any elevation of troponin T concentration in peripheral blood in patients undergoing CABG without CPB. The elevated troponin T level in the coronary sinus in 1 patient indicates cardiac release. However, since the elevation was present before the perioperative ischemia, this finding cannot be attributed to the LAD occlusion, but possibly to some minor ischemic episode in the preoperative period.

Cardiac C-fiber afferents are characterized by a rapid increase in firing rate on occlusion of a coronary artery.²² A proportion of these C-fibers contain CGRP, which is released by cardiac ischemia *in vitro*.¹⁹ Thus increased outflow of CGRP has been shown in the guinea pig heart after 5 minutes of ischemia.¹⁹ Furthermore, CGRP is released in the heart after activation of C-fiber afferents by lactic acid and low pH perfusion,²³ that is, conditions that correspond well with the metabolic disturbances associated with ischemia. CGRP has a cardioprotective effect in postischemic rat hearts, whereas the CGRP-receptor antagonist CGRP(8-37) impairs postischemic cardiac function.¹⁴ In the pig, *in vivo* local administration of CGRP augmented myocardial hyperemia after 45 minutes of LAD occlusion.²⁴

In previous studies in human beings, elevated levels of CGRP were found in peripheral blood in patients with acute myocardial infarction,¹⁵ but it could not be proved whether this represented myocardial release. The patients in the present study can be assumed to have some degree of collateral flow to the anterior wall, as opposed to patients who have an acute coronary arterial occlusion. The possible role for CGRP in such patients may be restricted to the ischemic border zone. The myocardial outflow of CGRP that is shown in this study implies that CGRP-mediated mechanisms of local vasodilation and myocardial protection found in animal studies may also be operative in myocardial ischemia in human beings.

The technical assistance by Gunilla Barr, CRNA, is gratefully acknowledged.

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