Endothelin Receptors in Endothelium-Denuded Human Coronary Artery Bypass Grafts and Coronary Arteries

Angelica Wackenfors, MS, Richard Ingemansson, MD, PhD, and Malin Malmsjö, MD, PhD

Departments of Internal Medicine and Cardiothoracic Surgery, Lund University Hospital, Lund, Sweden

Background. **Coronary artery bypass graft (CABG) surgery is hampered by deleterious vasospasm in the vessel wall, especially in vein grafts. Endothelin (ET) is a strong vasoconstrictor that can be observed in increasing concentrations during CABG surgery.**

Methods. **Endothelin-induced vasoconstriction was evaluated in isolated, endothelium-denuded vessel segments of the human saphenous vein (SV), left internal mammary artery (LIMA), and coronary arteries. The ET_A and ET_B receptor mRNA levels were quantified by real-time polymerase chain reaction (PCR) analysis.**

Results. **The ET_A and ET_B receptor mRNA levels were significantly higher in the SV than in the LIMA and the coronary arteries. ET-1 induced a more efficacious contraction in the SV and LIMA as compared with in the coronary arteries. The ET_B receptor agonist, Sarafotoxin 6c (S6c) stimulated constriction of the LIMA and SV, while inactive in the coronary arteries. The concentration-response curve for S6c was biphasic, suggesting activation of ET_A receptors at high concentrations as this response could be inhibited by FR139317 (10 μmol/L), and ET_B at low concentrations as this response could be inhibited by BQ788 (0.1 μmol/L).**

Conclusions. **Endothelin-induced vasoconstriction is mediated by ET_A receptors alone in coronary arteries, while a combination of ET_A and ET_B receptors are of importance in SV and LIMA. Expression of contractile ET_B receptors may be a pharmacologic disadvantage that contributes to the vasospasm during CABG surgery. The lower levels of ET_A and ET_B receptor mRNA in the LIMA and coronary arteries as compared with in the SV may provide one explanation for the better long- and short-term patency of LIMA as compared with SV grafts.**

(Ann Thorac Surg 2003;75:874–81)

Vasospasm is one cause of graft failure and morbidity in patients undergoing coronary artery bypass graft (CABG) surgery [1]. The etiology of graft vasospasm is likely to be multifactorial, including trauma at the time of surgery, disruption of the endothelium, and the presence of vasoactive substances. Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor [2]. Therefore, the role of endothelin peptides in the control of vascular tone in both physiology and pathophysiology is of interest. Plasma levels of ET-1 are elevated in patients undergoing CABG surgery [3], which implies that endothelin receptor antagonists may be therapeutic in reducing graft spasm, increasing the graft patency, and subsequently the outcome of CABG surgery.

Endothelin-1 binds to two different receptor subtypes: endothelin$_A$ (ET_A) at low concentrations and endothelin$_B$ (ET_B) at higher concentrations [4, 5]. The ET_A receptors are located on vascular smooth muscle cells and mediate vasoconstriction. The ET_B receptor mediates different responses depending on location. On endothelial cells, this G-protein coupled receptor induces an increase of cGMP and a subsequent release of dilatory mediators, while on smooth muscle cells the intracellular Ca^{2+} concentrations are increased leading to contraction. The ET_B receptors mainly induce endothelium-dependent dilatation by release of nitric oxide and prostaglandins [6]. During physiologic conditions the ET_B receptor mediated contractions are negligible [7]. Conversely during pathologic conditions such as arteriosclerosis [8], congestive heart failure [9], and subarachnoid hemorrhage [10] ET_B receptors are upregulated on smooth muscle cells and mediate vasoconstriction. ET_B receptor activity on smooth muscle cells may contribute to the pathologic vasomotor function and vasospasm during these conditions.

There are differences in the conducting characteristics of the two main coronary bypass vessels, the left internal mammary artery (LIMA) and the saphenous vein (SV). In the SV the occlusion rate amounts to 50% 10 years after CABG surgery mainly due to arteriosclerosis in the vein called "venous graft disease" [11]. In contrast the LIMA has good long-term patency [11]. Currently at the Lund University Hospital the LIMA is used as a graft to the left anterior descending coronary artery in 95% of the cases whereas the SV is used as a graft for the other occluded coronary arteries. The molecular mechanism responsible

Accepted for publication Oct 3, 2002.

Address reprint requests to A. Wackenfors, Division of Experimental Vascular Research, BMC A13, Lund University Hospital, SE-22184 Lund, Sweden; e-mail: angelica.wackenfors@med.lu.se.

Published by Elsevier Science Inc

0003-4975/03/$30.00
PII S0003-4975(02)04637-4

for the difference in pathologic development of the two grafts remains unknown. It is probable that the vasocontractile properties are of importance. In the present study the relative contribution of ET_A and ET_B receptors in mediating ET-induced vasoconstriction was evaluated in endothelium-denuded human coronary artery bypass grafts (the LIMA and the SV). The results were compared with that for endothelium-denuded, human coronary arteries, which has rarely been done before. In addition the ET_A and ET_B receptor mRNA levels in the LIMA, the SV and the coronary arteries were here quantified by real-time polymerase chain reaction (PCR) study.

Material and Methods

Tissue Collection

Coronary arteries were obtained from hearts that were explanted in the process of heart transplantation from 5 patients (4 male, 1 female; 26 to 57 years of age) suffering from congestive heart failure due to dilated cardiomyopathy. The LIMA and the SV were removed from 12 patients with coronary artery diseases (9 male, 3 female; 43 to 85 years of age) during CABG surgery. The vessels were removed from the patients and immediately immersed into cold buffer solution [14] and transported to the laboratory on dry ice. The vessels were immediately used for the experiments.

In Vitro Pharmacology

In the laboratory the vessels were dissected free from adhering tissue and the luminal side was gently rubbed with a metal wire to disrupt the endothelium. The vessels were then cut into cylindrical segments (3 to 4 mm long) and mounted on two L-shaped metal prongs, one of which was connected to a force displacement transducer for continuous recording of the isometric tension [12]. The mounted artery segments were immersed in temperature controlled (37°C), tissue baths containing a bicarbonate based buffer solution, which was continuously gassed with 5% CO_2 in O_2 resulting in a pH of 7.4. Eight to 16 ring segments were studied at the same time in separate tissue baths. The segments were allowed to stabilize at a resting tension of 4 mN (see results and [13]) for 1 hour before the experiments were started. The contractile capacity of each vessel segment was examined by exposure to a K^+ rich (63.5 mmol/L) buffer solution. Endothelium removal was assessed by exposure to acetylcholine (ACh), alternatively adenosine 5′-O-thiodiphosphate (ADPβS), which dilates blood vessels through an endothelium-dependent mechanism. Abolished dilatation indicated properly removed endothelium. For method details, see Malmsjö and associates [14].

Cumulative concentration-response curves were constructed for ET-1 and sarafotoxin 6c (S6c). Thereafter, the LIMA and the SV experiments were terminated by the addition of 10 μmol/L ACh and the coronary arteries, by the addition of 10 μmol/L ADPβS to check that the endothelium was removed. The ET-1 and S6c experiments were run in the absence (control) and presence of the selective ET_A antagonist, FR139317 (0.1 μmol/L to 10 μmol/L) or the selective ET_B antagonist BQ788 (0.1 μmol/L to 10 μmol/L) added 15 minutes before each agonist.

Length-Tension Measurements

The SV and the LIMA from 2 patients were removed and each vessel was divided into eight segments. The segments were immediately suspended in a bicarbonate based buffer solution and mounted for continuous recording of isometric tension (see above). The vessels were allowed to stabilize for 1 hour after which they were activated by a potassium-rich (63.5 mmol/L) buffer solution. After a short accommodation period (10 minutes) the distance between the metal prongs was increased, each time by 0.25 mm, and the tension recorded at different internal diameter. The tension was always allowed to reach a steady level between the extensions. The magnitude of the steady state tension was used in the calculations.

The optimal resting (wall tension) force in coronary arteries has been calculated using the same method before [13]. A resting force between 2 to 8 mN can be used in coronary arteries to obtain an optimal vascular response. In the present study the resting force of the coronary arteries was set to 4 mN.

Real-Time PCR

After removal of the endothelium, the vessels were snap frozen in liquid nitrogen and put in a −70°C freezer. Total cellular RNA was extracted using the FastRNA kit-green (Bio 101, Carlsbad, CA) following the suppliers' instructions. Reverse transcription of total RNA to cDNA was carried out using the GeneAmp RNA PCR kit (Perkin-Elmer, Applied Biosystems, Foster City, CA) in a Perkin-Elmer DNA thermal cycler. First strand cDNA was synthesized from 5 μg total RNA in a 100 μL reaction volume using random hexamers as primers.

Real-time PCR was performed in a GeneAmp 5700 sequence detection system (Perkin-Elmer, Applied Biosystems) using the GeneAmp SYBR Green kit (Perkin-Elmer, Applied Biosystems) with the cDNA synthesized above as template in a 50 μL reaction volume. The GeneAmp 5700 sequence detection system monitors the growth of DNA in real-time using an optic imaging system through the binding of a fluorescent dye to double-stranded DNA. Specific primers for the human ET_A and ET_B receptors were designed as follows: ET_A receptor forward: 5′-ATTGCCCTCAGCGAACAC-3′ reverse: 5′-CAACCAAGCAGAAAGACGGTC-3′. ET_B receptor forward: 5′-GATACGACAACTTCCGCTCCA-3′ reverse: 5′-GTCCACGATGAGGACAATGAG-3′

β-actin mRNA was used as a reference because it is the product of a housekeeping gene continuously expressed to a constant amount in cells. For method details, see Stenman and associates [15].

Drugs

The ET-1, S6c, FR139317, and BQ788, were purchased from Sigma (Stockholm, Sweden). All drugs were dissolved in 0.9% saline with 10% albumin. Oligonucleotides and reagents for the PCR assay were purchased from Perkin-Elmer (Applied Biosystems, Foster City, CA).

Calculations and Statistics

For in vitro pharmacology, all calculations and statistics were performed using GraphPad 3.02 software. E_{max} refers to the maximum contraction calculated as percent of the contractile capacity of 63.5 mmol/L K^+. The negative logarithm of the drug concentration that elicited 50% contraction (pEC_{50}) was determined by linear regression analysis using the values immediately above and below half-maximum response. When antagonists were used to inhibit the ET-1 and S6c contraction, the maximum contractile response was sometimes not reached within the agonist concentration interval and the real E_{max} value will therefore be similar to or higher than the obtained value. The pEC_{50} value was then calculated as the negative logarithm of the drug concentration at the contraction reaching 100% or 140% of K^+ (marked $pEC_{(100\%\ K+)}$ and $pEC_{(140\%\ K+)}$ in the text). The pharmacologic experiments were performed in arteries from 6 to 8 patients for each substance and statistical significance was accepted when p was less than 0.05, using Student's t test. All differences referred to in the text have been statistically verified. Values are presented as means ± SEM.

The contractile effect of S6c added in cumulative concentrations was analyzed according to a two-site model by fitting the following equation to the data by a nonlinear regression analysis:

$$E = E_{max}\left(\left(\frac{F_H \cdot [A]^{n_{H1}}}{EC_{50H}^{n_{H1}} + [A]^{n_{H1}}}\right) + \left(\frac{(1 - F_H) \cdot [A]^{n_{H2}}}{EC_{50L}^{n_{H2}} + [A]^{n_{H2}}}\right)\right).$$

In this equation, E_{max} denotes the overall maximum contractile response, F_H denotes the fraction of the response mediated by the high potency component, [A] denotes the agonist concentration, EC_{50H} and EC_{50L} denote the high and low potency EC_{50} values, and n_{H1} and n_{H2} denote the Hill coefficient of the high and low potency components, respectively.

For real-time PCR, 17 experiments were performed on the LIMA from 6 patients, 13 experiments on the SV from 6 patients, and 7 experiments in coronary arteries from 3 patients. The amount of ET_A and ET_B receptor mRNA was calculated as relative to the amount of β-actin mRNA in the same sample by the formula: $X_0/R_0 = 2^{CtR\text{-}CtX}$, where X_0 = original amount of ET receptor mRNA, R_0 = original amount of β-actin mRNA, CtR = C_T-value for β-actin and CtX = C_T-value for the ET receptor. Statistical analyses were performed using Student's t test, where p less than 0.05 was considered significant. All differences referred to in the text have been statistically verified.

Ethics

The project was approved by the Ethics Committee of Lund University in Sweden and conforms with the principles outlined in the Declaration of Helsinki.

Results

Length-Tension Measurements

The relationship between stepwise increments of the internal length and wall tension of vessel segments from the SV and the LIMA, one was kept in K^+-rich solution for induction of active tension (T_a) and the other that was kept in Ca^{2+}-free buffer for analysis of the passive tension (T_p), are shown in Figure 1A and 1B, respectively. A subtraction was made between the mean T_a and T_p ($T_a - T_p$) to visualize the greatest reactivity of the vessels. For the SV the optimal resting force was 5.2 mN (Fig 1A). A range from 3 to 6 mN can be used depending on the vessel size. For the LIMA the optimal resting force was calculated to be 4.3 mN (Fig 1B). A range from 3 to 5 mN can be used depending on vessel size. In the present study a resting tension of 4 mN was used in the SV and in the LIMA.

Potassium-Induced Contractions

The contractile response elicited by 63.5 mmol/L K^+ was 6.4 ± 1.6 mN in the coronary arteries, 2.2 ± 0.7 mN in the LIMA, and 3.4 ± 1.0 mN in the SV.

Endothelium Removal

After endothelium denudation vascular relaxations to 10 μmol/L ACh in the LIMA and SV and 10 μmol/L ADPβS in the coronary arteries were abolished, indicating that the endothelium was properly removed.

Contractile ET_A Receptors

Endothelin-1 induced a significantly more efficacious contraction in the LIMA and SV as compared with in the coronary arteries ($p < 0.05$; Fig 2A, Table 1), while the potency was similar (p = not significant; Fig 2A, Table 1). These results indicate higher amounts of functional ET_A receptors in the bypass vessels as compared with the coronary arteries. In accordance with this the level of ET_A receptor mRNA was higher in the SV as compared with in the LIMA and coronary arteries ($p < 0.05$; Fig 2B, Table 1).

In the presence of the selective ET_A receptor antagonist FR139317 (1 and 10 μmol/L), the ET-1 induced concentration-response curves were shifted to the right in a concentration-dependent manner in the LIMA (Fig 3, Table 1). This was also seen in the SV and in the coronary arteries (Table 1). These results indicate that ET-1 mainly induces contraction by activating ET_A receptors in the LIMA, the SV, and the coronary arteries.

Contractile ET_B Receptors

The ET_B receptor agonist S6c induced vasoconstriction in 58% of the SV and LIMA, while inactive in the coronary arteries. The S6c concentration-response curves were

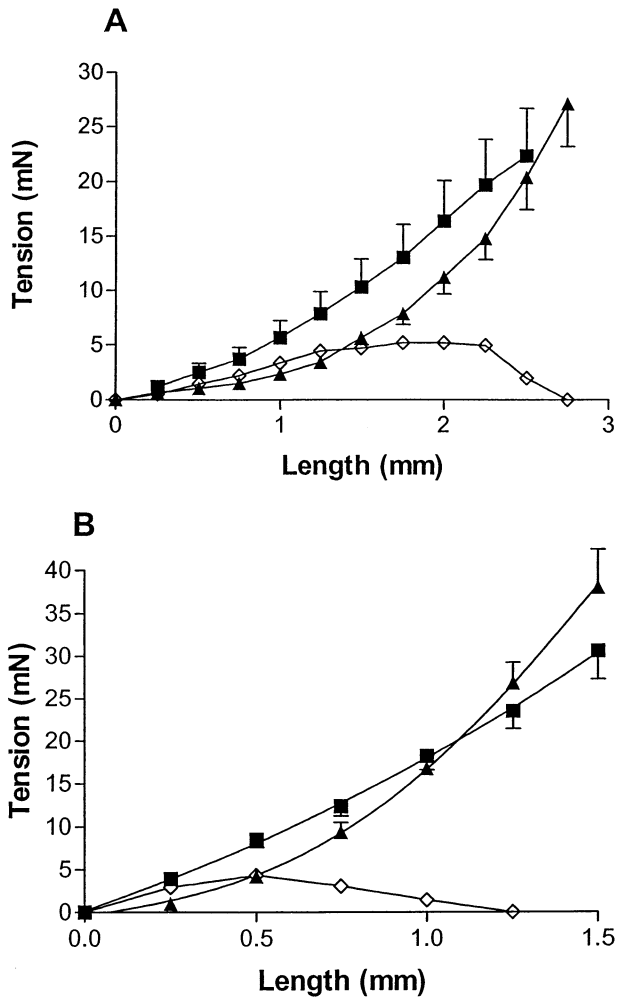

Fig 1. Relation between effective vessel lumen length and wall tension in relaxed (T_p; triangles*) and K^+ activated (T_a;* squares*) (A) human saphenous vein* (SV) *and (B) left internal mammary artery (LIMA). The T_p at the various lengths was derived from vessels immersed in Ca^{2+}-free buffer solution. The vessels were stepwise extended from slack length to a length of 3 mm for the SV and 1.5 mm for the LIMA and the wall tension measured at each length. Separate vessel segments were initially contracted by K^+ before being subjected to identical stretches and the active tension (T_a) was recorded. A subtraction was made between the mean T_a and T_p ($T_a - T_p$;* diamonds*) to visualize the greatest reactivity of the vessels. Data are shown as means of eight measurements and presented as mean values ± SEM.*

constructed from the active vessel segments. In the SV and in the LIMA the S6c concentration-response curves were biphasic (Fig 4A). The selective ET_B receptor antagonist BQ788 (0.1 μmol/L) inhibited the first part of the S6c concentration-response curve in the LIMA ($p < 0.05$; Fig 4B, Table 1), indicating that this part of the response was mediated by ET_B receptors. The pEC_{50} value and Hill-slope of the second part of the S6c concentration-response curve was similar to that for the ET_A receptor agonist, ET-1, in the LIMA (p = not significant; Table 1). Likewise the Hill-slope was 0.9 for S6c and 1.1 for ET-1 (p = not significant). In addition the second part of the S6c concentration-response curve was inhibited by FR139317 (Fig 4C, Table 1), indicating that it was mediated by ET_A receptors.

Sarafotoxin 6c, an ET_B receptor agonist, did not induce constriction of the coronary arteries, suggesting absence of functional ET_B receptors. The first part of the S6c-induced constriction was more efficacious in the SV as compared with the LIMA ($p < 0.05$; Fig 4A, Table 1), which is in accordance with the real-time PCR experiments that showed significantly higher levels of ET_B receptor mRNA in the SV as compared with the LIMA and the coronary arteries ($p < 0.05$; Fig 5).

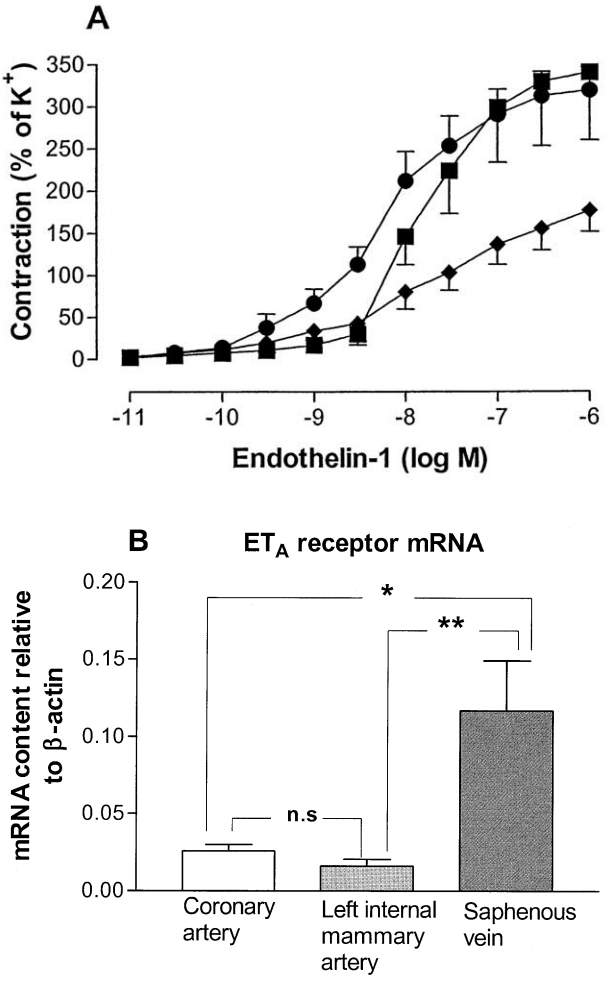

Fig 2. (A) Concentration-response curves to endothelin-1 in human coronary arteries (diamonds*), the left internal mammary artery ([LIMA]* squares*) and the saphenous vein ([SV]* circles*). Vasoconstriction is expressed as a percentage of the maximal contraction induced by 63.5 mmol/L K^+, and presented as mean values ± SEM. (B) Endothelin$_A$ receptor mRNA levels in the left internal mammary artery, saphenous vein, and coronary arteries were assessed by real-time polymerase chain reaction study. Values are presented as mean values ± SEM, calculated as percent of the β-actin levels. Statistical analyses were performed using Student's* t *test.* *p < 0.05 **p < 0.01.

Table 1. Contractile Responses to ET-1 and S6c, With and Without ET_A Receptor Antagonist FR139317 and ET_B Receptor Antagonist BQ788, in the LIMA, SV, and Coronary Arteries

	E_{max} (%)	pEC_{50} (− log mol/L)	$pEC_{140\%K+}$ (− log mol/L)	$pEC_{100\%K+}$ (− log mol/L)
LIMA				
ET-1	341 ± 6	7.8 ± 0.1	8.1 ± 0.1	
ET-1 + FR139317 (1 μmol/L)	—	—	6.9 ± 0.1	
ET-1 + FR139317 (10 μmol/L)	—	—	6.1 ± 0.1	
S6c; 1st response	35 ± 1	9.5 ± 0.1		
S6c + BQ788 (0.1 μmol/L)	31 ± 3	6.4 ± 0.1		
S6c; 2nd response	171 ± 6	7.2 ± 0.1		7.4 ± 0.1
S6c + FR139317 (10 μmol/L)	—	—		6.0 ± 0.1
SV				
ET-1	322 ± 5	8.3 ± 0.1	8.7 ± 0.1	
ET-1 + FR139317 (1 μmol/L)	—	—	7.3 ± 0.1	
S6c; 1st response	73 ± 1	9.2 ± 0.1		
S6c + BQ788 (0.1 μmol/L)	72 ± 3	7.8 ± 0.1		
Coronary artery				
ET-1	200 ± 6	7.6 ± 0.1		7.6 ± 0.2
ET-1 + FR139317 (10 μmol/L)	—	—		6.4 ± 0.1
S6c	0 ± 0	—		

The responses are expressed as percentage of an initial contraction by 63.5 mmol/L K^+. Data are shown as E_{max} or pEC_{50} ± SEM. When E_{max} was not reached within the agonist-concentration interval, the pEC_{50} value was then calculated as the negative logarithm of the drug concentration at a the contraction reaching 100% or 140% of K^+ (marked $pEC_{(100\%\ K+)}$ and $pEC_{(140\%\ K+)}$) in the table.

ET-1 = endothelin-1; LIMA = left internal mammary artery; S6c = sarafotoxin 6c; SV = saphenous vein.

Comment

In the present study ET receptor mRNA levels were measured by real-time PCR in endothelium-denuded human coronary bypass grafts and then compared with those in human coronary arteries. The ET_A and ET_B receptor mRNA levels were higher in the SV than in the LIMA and coronary arteries. The ET receptors on vascular smooth muscle cells are both mitogenic, contributing to the development of arteriosclerosis, and mediate strong vasoconstriction, which may lead to vasospasm and hypoperfusion commonly observed during CABG surgery where the circulating ET levels are elevated [1, 3, 16]. The low ET receptor mRNA levels in LIMA may provide one explanation for the better long- and short-term patency of LIMA as compared with SV grafts. In vitro pharmacology experiments in endothelium-denuded vessels revealed that ET elicit vasoconstriction by activating ET_A receptors alone in coronary arteries whereas a combination of ET_A and ET_B receptors are of importance in the SV and LIMA. Expression of contractile ET_B receptors may be a pharmacologic disadvantage that contributes to vasospasm during CABG surgery.

Before the experiments were started the endothelium was removed mechanically to minimize the influence that a varying endothelium function would imply in these arteries from patients with coronary artery disease and heart failure. Previous results from our group have shown that the endothelium function is poor in human coronary arteries that are obtained during the procedure of heart transplantation. The arteries did not dilate when exposed to 1 μmol/L ACh, probably owing to coronary artery disease [17].

ET_A Receptor Mediated Vasoconstriction

The ET_A receptor agonist, ET-1, induced a significantly more efficacious contraction in the coronary bypass grafts as compared with in the coronary arteries (Fig 2A). Endothelin-1 plasma levels are 1 to 5 pmol/L in healthy humans. In vascular disease the plasma concentration of ET-1 is increased from picomolar to nanomolar concentrations [18]. During CABG surgery the ET-1 plasma levels are further elevated [3]. High ET-1 plasma levels in combination with the greater effect of ET-1 in the coronary bypass grafts increase the risk of efficacious vasocontractile effects. Consequently ET-1 may be a cause of the deleterious vasospasm that is commonly observed in the coronary bypass grafts during surgery [1].

When the two different bypass grafts were compared ET-1 could be shown to induce a significantly more potent vasoconstriction in the SV as compared with in the LIMA (Fig 2A). Furthermore ET_A receptor mRNA levels were higher in the SV (Fig 2B). The ET_A receptors mediate strong vasoconstriction, which may provide one explanation of why vein grafts are more susceptible to the development of spasm than artery grafts [11]. On the other hand the lumen of the SV is larger than of the LIMA and vasospasm in the SV may therefore have less deleterious consequences for the patient. Further well-documented, potentially harmful effects of ET-1 include proliferation of vascular smooth muscle cells and the subsequent development of arteriosclerosis [16]. The high ET_A receptor mRNA levels in the SV in contrast to in the LIMA, in combination with the elevated levels of ET-1 in heart failure patients, may contribute to the rapid

development of arteriosclerosis and long-term failure of vein grafts called "venous graft disease" [11, 19].

In the presence of the selective ET_A receptor antagonist FR139317 the concentration-response curve for ET-1 was shifted to the right in the LIMA, SV, and coronary arteries, which is in accordance with the belief that ET-1 induces vasoconstriction by activating ET_A receptors [20–22].

ET_B Receptor Mediated Vasoconstriction

Sarafotoxin 6c is believed to be a selective ET_B receptor agonist [23]. Sarafotoxin 6c induced vasoconstrictions in the endothelium denuded SV and LIMA. When the Hill-slope was calculated for the entire S6c concentration-response curve it was found to be 1.9 for the SV and 3.2 for the LIMA. Simple binding is supposed to be hyperbolic and thus have a Hill-slope of one. When an agonist activates a multitude of receptors or second messengers, the slope of the curve will alter [24]. A closer look at the concentration-response curve revealed a biphasic tendency suggesting involvement of two different types of receptors: ET_B at low concentrations and ET_A at high concentrations. This conclusion was further supported in that the pEC_{50} value and the Hill-slope for the second part of the S6c concentration-response curve were similar to that for the ET_A receptor agonist, ET-1. Furthermore the selective ET_A receptor antagonist FR139317 (0.1 μmol/L) could antagonize this response. A closer look at published S6c concentration-response curves revealed biphasic properties that were not discussed [25]. It has recently been suggested that ET_A receptors play a role in mediating the systemic vasoconstrictor response to high doses of S6c, further supporting our results [26]. Previous reverse transcriptase (RT)-PCR has proven that both ET_A and ET_B receptors are located on the smooth muscle cells of the SV and LIMA and also ligand-binding studies have demonstrated the presence of these receptors in vascular smooth muscle [20]. By real-time PCR experiments in this study, the presence of ET_A and ET_B receptor mRNA in smooth muscle cells from the SV and the LIMA could be verified. Coronary arteries did not contract when exposed to S6c. Thus there are no functional ET_B receptors. The levels of ET_A receptor mRNA were low in the coronary arteries and were probably not stimulated by the concentrations of S6c used in the present experiments.

The SV and LIMA from 42% of the patients did not respond to S6c. Others have observed the same irregularity in the responses [23]. The expression of contractile ET_B receptors thus varies greatly. The cause of this variation is not known although the blood vessels derive from patients with different diseases. The coronary arteries were obtained from hearts that were explanted in the procedure of heart transplantation. These patients suffered from congestive heart failure due to dilated cardiomyopathy. The LIMA and SV were obtained from patients who suffered from coronary artery disease due to arteriosclerosis. In arteriosclerosis [8] and coronary artery disease [27] contractile ET_B receptors are upregulated and appear on vascular smooth muscle. Different

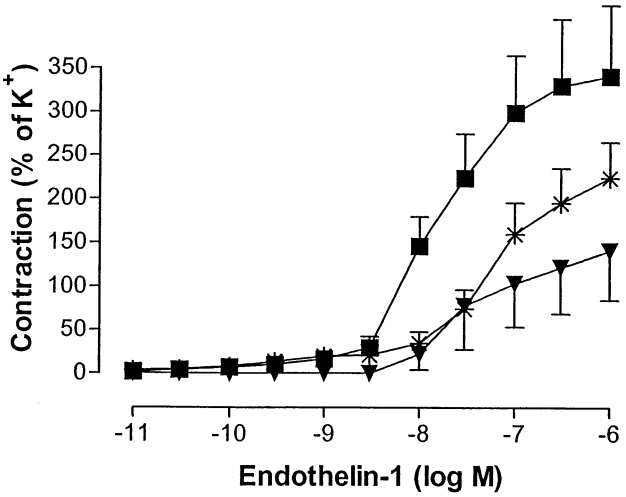

Fig 3. Endothelin-1 induced vasoconstriction in the left internal mammary arteries in the absence and presence of the selective endothelin$_A$ receptor antagonist FR139317. Vasoconstriction is expressed as a percentage of the maximal contraction induced by 63.5 mmol/L K^+, and presented as mean values ± SEM. Squares = *control;* asterisks = *FR139317 1 μmol/L;* triangles = *FR139317 10 μmol/L.*

degrees of arteriosclerosis or different types of atherosclerotic disease may be responsible for the variations seen in the vasocontractile responses to S6c in the present experiments [20].

In physiologic conditions ET_B receptors mediate endothelium-dependent dilatation through the release of nitric oxide and prostaglandins [6]. Conversely, in arteriosclerosis, congestive heart failure, and subarachnoid hemorrhage ET_B receptors are upregulated on smooth muscle cells and elicit vasoconstriction [8–10]. ET_B receptor activity on smooth muscle cells may contribute to the pathologic vasomotor function and vasospasm during these conditions. S6c-elicited vasoconstriction and ET_B receptor mRNA expression could be shown in the bypass grafts, LIMA and the SV. Conversely, no pharmacologic activity of S6c could be noted in the coronary arteries. Contractile ET_B receptors on the smooth muscle cells of the bypass grafts may be a pharmacologic disadvantage that contributes to the vasospasm during CABG surgery. Despite the absence of S6c effect in the coronary arteries, a small amount of ET_B receptor mRNA could be found by real-time PCR. The reason for this discrepancy is not known although mRNA expression for a receptor does not necessarily mean that there is active receptor protein on the cell surface.

Conclusion

Bypass surgery is hampered by the deleterious vasospasm in the conduit vessels. Many measures have been undertaken to control the vessel tone during surgery including dilating the vessels with potent vasodilators such as papaverine, sodium nitroprusside, and nifedipine and distending the vessel with NaCl [28, 29]. Circulating plasma levels of ET are elevated in heart failure patients and during CABG surgery [3, 19]. One difference

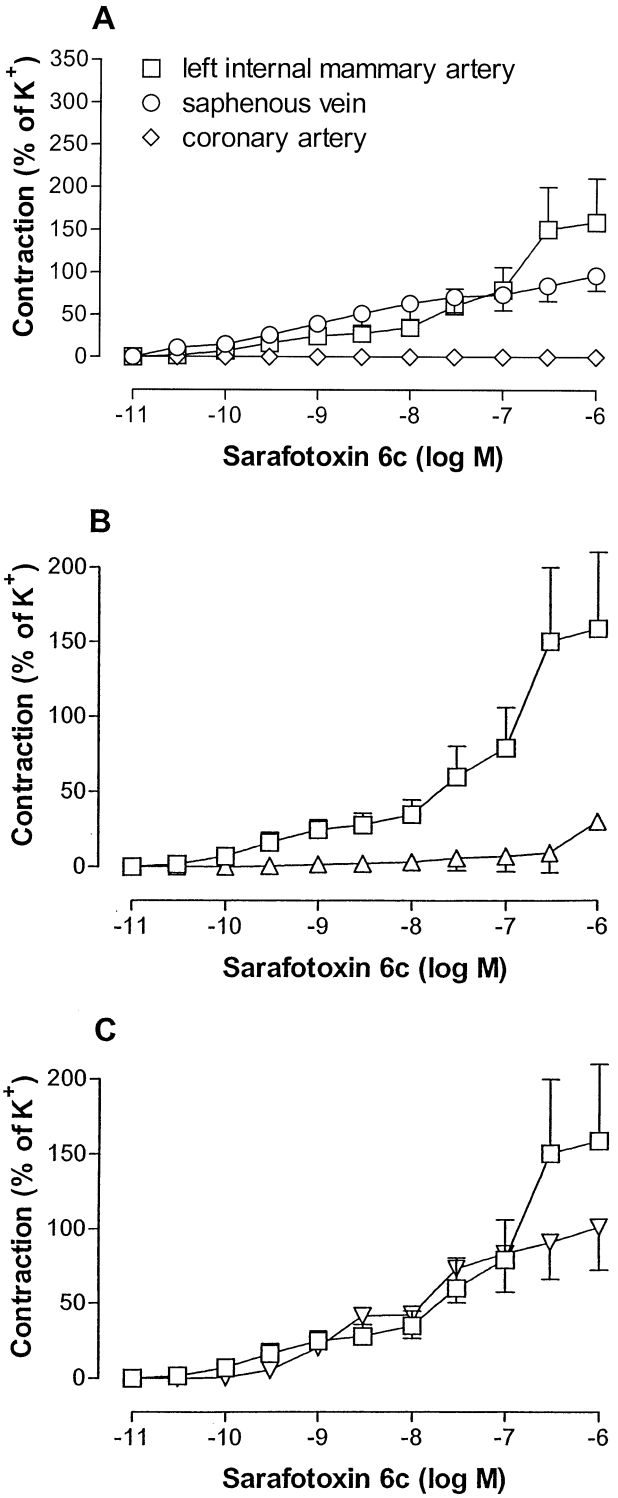

Fig 4. (A) Concentration-response curves to sarafotoxin 6c in human coronary arteries (diamonds)*, the left internal mammary artery* (squares)*, and the saphenous vein* (triangles; squares = *control). (B) The first part of the sarafotoxin 6c contractile response was inhibited by 0.1 μmol/L BQ788. (C) The second part of the sarafotoxin 6c contractile response was antagonized by 10 μmol/L FR139317. Vasoconstriction is expressed as a percentage of the maximal contraction induced by 63.5 mmol/L K^+ and is presented as mean values ± SEM.*

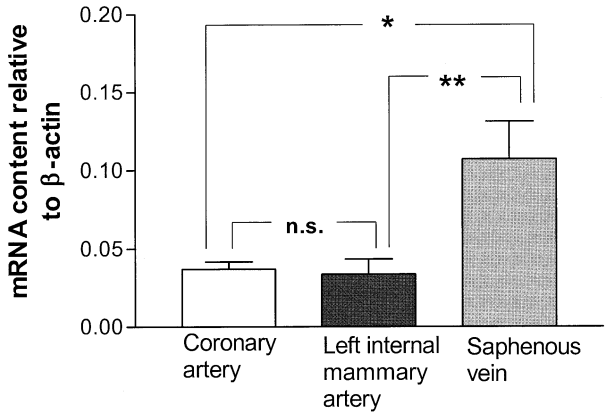

Fig 5. Endothelin$_B$ receptor mRNA levels in the left internal mammary artery, saphenous vein and coronary arteries were assessed by real-time polymerase chain reaction study. Values are presented as mean values ± SEM, calculated as percent of the β-actin levels. Statistical analyses were perfor;med using Student's t *test.* $^*p < 0.05$ $^{**}p < 0.01$.

between the bypass grafts and the coronary arteries is that both ET_A and ET_B receptors mediate the vasoconstrictor effects of ET in bypass grafts, while only ET_A is of importance in coronary arteries. Contractile ET_B receptors may be a pharmacological disadvantage that contributes to vasospasm during CABG surgery. Furthermore the ET_A and ET_B receptor mRNA levels are significantly higher in the SV as compared with the LIMA and coronary arteries, which may explain the higher frequency of vasospasm in the vessel wall at the time of surgery in SV and restenosis due to "venous graft disease." An ET receptor antagonist could be of use in preventing perioperative graft vasospasm and postoperative restenosis. Because not only ET_A but also ET_B receptors mediate strong vasoconstriction in the bypass grafts, an ET_A/ET_B balanced antagonist might have beneficial effects. Further knowledge about the molecular basis of the vasospasm will hopefully provide us with pharmacologic tools in the future to revoke the spasm during surgery.

Limitation of the Study

It should be noted that this study was performed on coronary arteries from patients suffering from congestive heart failure due to dilated cardiomyopathy and on LIMA and SV from patients with coronary artery disease. The coronary arteries were used as a control and for obvious reasons it is impossible to obtain coronary arteries from a patient undergoing CABG surgery.

This study was supported by the Crafoord Foundation, the Swedish Hypertension Society, the Royal Physiographic Society (Lund), and the Swedish Research Council (grant no. 5958).

References

1. Rosenfeldt FL, He GW, Buxton BF, Angus JA. Pharmacology of coronary artery bypass grafts. Ann Thorac Surg 1999;67: 878–88.
2. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–5.
3. Haak T, Matheis G, Kohleisen M, Ngo H, Beyersdorf F, Usadel KH. Endothelin during cardiovascular surgery: the effect of diltiazem and nitroglycerin. J Cardiovasc Pharmacol 1995;26(Suppl 3):S494–6.
4. Arai H, Hori S, Aramori I, Ohkubo H, Nakanishi S. Cloning and expression of a cDNA encoding an endothelin receptor. Nature 1990;348:730–2.
5. Sakurai T, Yanagisawa M, Takuwa Y, et al. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature 1990;348:732–5.
6. de Nucci G, Thomas R, D'Orleans-Juste P, et al. Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium-derived relaxing factor. Proc Natl Acad Sci USA 1988;85:9797–800.
7. Moller S, Edvinsson L, Adner M. Transcriptional regulated plasticity of vascular contractile endothelin ET(B) receptors after organ culture. Eur J Pharmacol 1997;329:69–77.
8. Dagassan PH, Breu V, Clozel M, et al. Up-regulation of endothelin-B receptors in atherosclerotic human coronary arteries. J Cardiovasc Pharmacol 1996;27:147–53.
9. Cannan CR, Burnett JC Jr, Lerman A. Enhanced coronary vasoconstriction to endothelin-B-receptor activation in experimental congestive heart failure. Circulation 1996;93:646–51.
10. Roux S, Loffler BM, Gray GA, Sprecher U, Clozel M, Clozel JP. The role of endothelin in experimental cerebral vasospasm. Neurosurgery 1995;37:78–86.
11. Steinbruchel DA. Arterial revascularization: evidence or presumption? Scand Cardiovasc J 2000;34:463–7.
12. Högestätt ED, Andersson KE, Edvinsson L. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand 1983;117:49–61.
13. Saetrum Opgaard O, Edvinsson L. Mechanical properties and effects of sympathetic co-transmitters on human coronary arteries and veins. Basic Res Cardiol 1997;92:168–80.
14. Malmsjö M, Adner M, Harden TK, Pendergast W, Edvinsson L, Erlinge D. The stable pyrimidines UDPbetaS and UTPgammaS discriminate between the P2 receptors that mediate vascular contraction and relaxation of the rat mesenteric artery. Br J Pharmacol 2000;131:51–6.
15. Stenman E, Malmsjö M, Uddman E, Gidö G, Tadeuz W, Edvinsson L. Cerebral ischemia upregulates vascular endothelin ET_B receptors in rat. Stroke 2002. In press.
16. Nakaki T, Nakayama M, Yamamoto S, Kato R. Endothelin-mediated stimulation of DNA synthesis in vascular smooth muscle cells. Biochem Biophys Res Commun 1989;158:880–3.
17. Holmgren A, Pantev E, Erlinge D, Edvinsson L. Inhibition of angiotensin II-induced contraction by losartan in human coronary arteries. J Cardiovasc Pharmacol 1998;32:662–4.
18. Hansen-Schwartz J, Edvinsson L. Increased sensitivity to ET-1 in rat cerebral arteries following organ culture. Neuroreport 2000;11:649–52.
19. Love MP, Ferro CJ, Haynes WG, et al. Endothelin receptor antagonism in patients with chronic heart failure. Cardiovasc Res 2000;47:166–72.
20. Maguire JJ, Davenport AP. ETA receptor-mediated constrictor responses to endothelin peptides in human blood vessels in vitro. Br J Pharmacol 1995;115:191–7.
21. Maguire JJ, Kuc RE, O'Reilly G, Davenport AP. Vasoconstrictor endothelin receptors characterized in human renal artery and vein in vitro. Br J Pharmacol 1994;113:49–54.
22. Aramori I, Nirei H, Shoubo M, et al. Subtype selectivity of a novel endothelin antagonist, FR139317, for the two endothelin receptors in transfected Chinese hamster ovary cells. Mol Pharmacol 1993;43:127–31.
23. Sokolovsky M. Endothelins and sarafotoxins: receptor heterogeneity. Int J Biochem 1994;26:335–40.
24. Leff P, Prentice DJ, Giles H, Martin GR, Wood J. Estimation of agonist affinity and efficacy by direct, operational model-fitting. J Pharmacol Methods 1990;23:225–37.
25. Möller S, Edvinsson L, Adner M. Transcriptional regulated plasticity of vascular contractile endothelin ET(B) receptors after organ culture. Eur J Pharmacol 1997;329:69–77.
26. Rasmussen TE, Jougasaki M, Supaporn T, Hallett JW Jr, Brooks DP, Burnett JC Jr. Cardiovascular actions of ET-B activation in vivo and modulation by receptor antagonism. Am J Physiol 1998;274:R131–8.
27. Wenzel RR, Duthiers N, Noll G, Bucher J, Kaufmann U, Luscher TF. Endothelin and calcium antagonists in the skin microcirculation of patients with coronary artery disease. Circulation 1996;94:316–22.
28. LoGerfo FW, Haudenschild CC, Quist WC. A clinical technique for prevention of spasm and preservation of endothelium in saphenous vein grafts. Arch Surg 1984;119:1212–4.
29. Roubos N, Rosenfeldt FL, Richards SM, Conyers RA, Davis BB. Improved preservation of saphenous vein grafts by the use of glyceryl trinitrate-verapamil solution during harvesting. Circulation 1995;92(Suppl 2):31–6.