Circumferential stretching of saphenous vein smooth muscle enhances vasoconstrictor responses by Rho kinase-dependent pathways

Emma McGregor, Martin Gosling, David K. Beattie, Duncan M.P. Ribbons, Alun H. Davies, Janet T. Powell

Abstract

Objective: Surgical preparation and/or pulsatile arterial perfusion of saphenous vein increases the sensitivity of vein rings to calcium mobilising agonists such as phenylephrine. We have investigated the mechanism(s) underlying this effect. Methods: We have used an ex vivo flow circuit, with simulated arterial or venous flows (mean pressure 100 and 20 mmHg, respectively), to investigate the sensitivity of human saphenous vein to phenylephrine, 5-hydroxytryptamine (5-HT) and KCl, using organ chamber pharmacology. Results: After 90 min of pulsatile arterial perfusion the mean maximum tension induced by KCl had increased from 4.7 to 11.1 g (n=5), by phenylephrine had increased from 4.4 to 10.2 g (n=8) and by 5-HT had increased from 4.4 to 6.7 g (n=10), all P<0.01. Phenylephrine did not augment the tension in vein rings maximally precontracted with KCl (n=4). The EC_{50} for KCl was unchanged after pulsatile arterial perfusion (n=5), but for phenylephrine and 5-HT there were significant reductions from 14±5 to 2±1 µM (n=8) and from 1.0±0.4 to 0.20±0.06 µM (n=10), respectively. The rate of contraction (in response to 3 µM phenylephrine) increased from 0.11 g/min to 0.37 g/min, P<0.02, after arterial perfusion (n=4). These changes in contractile properties (to phenylephrine) were endothelium-independent, evident within 5 min of simulated arterial perfusion. The changes in contractile properties could be abrogated by external stenting of the vein (to attenuate circumferential deformation) or inclusion in the perfusate of a vasodilator, e.g., cromakalim (5 µM) or the selective Rho kinase inhibitor Y-27632 (20 µM). The heightened sensitivity and contractility to phenylephrine was maintained after inclusion of adenosine (100 µM), gadolinium (10 µM) or cycloheximide (10 µM) in the vein perfusate. Conclusions: The circumferential deformations imposed by simulated arterial perfusion alter the vasomotor responses of saphenous vein smooth muscle. These effects are independent of new protein synthesis or the activation of stretch activated cation channels. The Rho kinase pathway appears to mediate the signalling mechanisms leading to increased agonist-induced tension and the increased sensitivity to vasoconstrictors. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Cardiovascular surgery; Contractile function; Protein kinases; Smooth muscle; Veins

1. Introduction

When saphenous vein is used as an arterial bypass conduit, the vessel experiences an abrupt change from the low pressure, minimally pulsatile venous circulation to the high pressure, pulsatile arterial circulation. In arteries, medial smooth muscle cells are organised circumferentially and spirally in a manner suited to conducting the arterial pulse. In contrast, the smooth muscle cells of saphenous vein are oriented both longitudinally and circumferentially. The re-orientation of the medial smooth muscles of saphenous vein into co-ordinated circumferential and spiral units is a crucial adaptive response observed in vein grafts. Another adaptive change is the development of intimal hyperplasia. This results from migration of smooth muscle cells into the intima, where these cells proliferate and elaborate a dense connective tissue matrix. These well-described histological changes represent the chronic...
adaptation of saphenous vein to the arterial circulation. Much less is known about the early responses of saphenous vein smooth muscle to arterial pressure and flow.

Saphenous vein has an active myogenic response, contracting in response to acute increases in luminal pressure [1]. Additionally, saphenous vein prepared for grafting or exposed to arterial flow conditions rapidly acquires an increased sensitivity to phenylephrine and catecholamines, which increases the tension of smooth muscle [2–4]. The synergistic effect of these responses might predispose to graft vasospasm as well as exacerbating the hypoxic and metabolic changes in the medial smooth muscle of newly implanted vein grafts [5,6]. In clinical practice grafts are treated with papaverine or overdistended to minimise vasospasm [7]. Our hypothesis is that the early changes in vein contractility result from altered intracellular signalling pathways, which may be coupled to the proliferative changes leading to the exaggerated intimal hyperplasia, which causes vein graft stenosis and failure. It is well established that vasoconstrictors, including angiotensin II and phenylephrine, stimulate the proliferation of cultured smooth muscle cells, although these cultured cells are no longer contractile [8].

The forces imposed on a newly implanted vein graft include shear stress, radial, circumferential and longitudinal deformations [9]. We have developed an experimental model for exposing saphenous vein to arterial flow dynamics in vitro, in which the circumferential and radial deformations can be attenuated by an external polytetrafluoroethylene (PTFE) stent [10]. Interestingly, external stenting of vein has been demonstrated to increase the luminal diameter and reduce intimal hyperplasia in experimental vein grafts [11,12].

Here, we have used pharmacological and mechanical interventions to identify the signalling mechanisms underlying the early responses of saphenous vein smooth muscle to arterial haemodynamics.

2. Methods

2.1. Patients

Saphenous vein was harvested from patients undergoing aortocoronary (n=98) or infragenual bypass (n=8), amputation (n=5) or high ligation of saphenous vein for correction of varicose veins (n=33), with consent and the approval of the local Ethical Committee. The vein was transported to the laboratory in ice-cold Krebs solution. Diseased vein, which did not respond to phenylephrine (10 μM) with a contraction of >1 g was discarded. Samples from current smokers and patients with diabetes were excluded.

2.2. Materials

Modified Krebs’ solution (in mmol/l NaCl 118.4, KCl 4.7, KH2PO4 1.2, MgSO4·7H2O 1.2, glucose 11.1, NaHCO3 24.9, CaCl2 2.5) was made freshly each day. Calcium ionophore (A23187), phenylephrine, 5-HT, sodium nitroprusside, cromakalim and cycloheximide were obtained from Sigma. Stock solutions of cromakalim were made in ethanol. The polytetrafluoroethylene stents were a gift from Impra (UK). The Rho kinase inhibitor Y-27632 was a gift from Welfide, Japan.

2.3. The flow circuit

Saphenous vein (3–4 cm) was mounted in a retaining jig, after removal of a control section of vein for histology, and organ bath studies. The jig was then placed in a bypass circuit consisting of a perfusion pump (Stockert, Munich, Germany) two water baths (one to warm the circulating Krebs reservoir and one to warm the stationary solution surrounding the adventitia of the vein), the circuit apparatus and a pressure transducer (S & W Medico Teknik, Albertslund, Denmark). The internal diameter of the vein was monitored using both B- and M-mode ultrasonography (Aloka SSD-500 with a 7.5-MHz linear transducer), through a plastic viewing panel 1.6 cm above the submerged vein, at 15-min intervals during the experiment. Veins were perfused with oxygenated Krebs solution at 36.5°C (oxygen content 20 ml/l). Veins were exposed either to pulsatile flow (90 cpm) at a mean pressure of 100 mmHg (arterial flow rate 200–225 ml/min, maximum/minimum pressure 120/80 mmHg, calculated shear stress 0.26±0.09 N/m²), or to non-pulsatile flow at 20 mmHg (venous flow rate 10–20 ml/min, calculated shear stress 0.021±0.011 N/m²). The shear stress was estimated, assuming laminar flow in a cylindrical tube by the formula: shear stress = 32(flow rate×fluid viscosity)/π(diameter³).

The dilation of vein, measured by ultrasonography, in response to arterial pressures attenuates the increase in shear stress. Some veins were placed inside a tube (2–4 cm in length) of externally supported polytetrafluoroethylene (PTFE), which was non-restrictive, but sized to limit circumferential distension of the vein during arterial flow (calculated shear stress 0.40±0.13 N/m²). In some experiments the Krebs solution perfusing the vein was supplemented with cromakalim (5 μM), sodium nitroprusside (20 μM) or cycloheximide (10 μM).

2.4. Organ chamber studies

Vein rings, 5 mm in length, were mounted in a 10-ml organ chamber suspended between two 0.2-mm steel wire stirrups, the upper one being attached to an isometric force transducer. Vein rings were stretched at 0.5 g intervals until the optimum-length tension relationship was obtained, usually 1.5–2 g. The changes in tension generated by the rings were recorded in both electronic format (MacLab Software) and a hard copy (Student Oscillograph, Harvard Apparatus, Kent). Responses to phenylephrine, 5-HT, KCl,
A23187, and sodium nitroprusside were determined as described previously [13]. Relaxation in response to A23187 was used to confirm the presence of functional endothelium. Similar studies were performed for vein rings denuded of endothelium by passing a fine wire firmly across the luminal surface: removal of endothelium was confirmed by histology [13]. Relaxations are reported as a percentage of the relaxation observed with 20 μM sodium nitroprusside.

2.5. Medial dissection from human saphenous vein

Connective tissue was removed from vein segments (1–2 cm) and the lumen opened longitudinally. Endothelium was removed by scraping the lumen with a scalpel and washing with PBS (pH 7.2). Using forceps the media was peeled from the adventitia. Dissected tissue was immediately frozen between aluminium tongs (pre-cooled in liquid nitrogen) and samples stored at −70°C.

2.6. Preparation of medial smooth muscle lysates for Western blotting/slot blotting

Using a ceramic pestle and mortar, cooled at −70°C, frozen tissue samples (70–110 mg) were pulsed with a fine powder, under liquid N₂. The powder was homogenised in lysis buffer (10 mM Tris–Cl, pH 7.4, 140 mM NaCl, 1 mM phenylmethylsulphonyl fluoride, 1 mM NaVO₃, 0.8 U/ml aprotinin, 1 mM EDTA, 2.9 μM pepstatin A, 4.68 μM leupeptin, 1% (v/v) NP40) in 1.5 ml Eppendorf tubes, sonicated at 4°C for 5 min and centrifuged at 10,000×g at 20°C for 5 min. The protein concentration in the supernatant was determined and a 30-μg protein load was used for both Western blots (12% acrylamide SDS–PAGE) and slot blots.

Slot blotting onto PVDF Immobilon-P transfer membrane (Millipore), was carried out under vacuum using a Bio-Dot SF Microfiltration apparatus (Bio-Rad, Herts, UK). Membrane was set up according to manufacturers instructions and serial dilutions of medial smooth muscle lysate (200 μl) were applied to the centre of each sample well. GAPDH was detected using a monoclonal antibody (Chemicon) at a 1:1000 dilution and MLCK using a monoclonal antibody (Sigma) at a 1:1000 dilution. A horseradish peroxidase-conjugated secondary antibody (Dako) was used at a 1:2500 dilution for both MLCK and GAPDH. Proteins were detected using enhanced chemiluminescence.

2.7. Analysis of data

Differences in ATP concentrations were compared by Student’s paired t-test. Relaxation of vein rings was reported as a percentage of the contraction to phenylephrine (mean±S.E.M.). The 50% effective concentrations (EC₅₀) of the drugs used were calculated by normalising data to the peak response and fitting the concentration–response curve to a logistic plot incorporating Hill coefficient using MicroCal Origin (MicroCal, Northampton, MA, USA). Evaluation of this data was performed using the student’s t-test for paired-observations or for concentration–response curves using repeated measures analysis of variance, followed by Bonferroni multiple comparison test (Statview 4.0 for Macintosh).

3. Results

3.1. Altered responsiveness of vein to phenylephrine, 5-HT and KCl after arterial flow conditions

After saphenous vein was exposed to arterial flow conditions for 90 min the EC₅₀ for phenylephrine decreased from 14±5 to 2±1 μM, P<0.01 (Table 1) and the maximum tension increased from 4.4±0.4 to 10.2±1.1 g, P<0.05 (n=8). This heightened sensitivity and contractility to phenylephrine occurred before and after endothelial denudation and was observed as early as after 5 min of arterial flow conditions (EC₅₀ 3±1 μM). In contrast, after 90 min of venous flow conditions the EC₅₀ for phenylephrine (9±1 μM) was unchanged (Table 1). The rate of contraction, in response to 1 μM phenylephrine, increased from 0.03 g/min before arterial perfusion to 0.3 g/min after arterial perfusion, P<0.03 (n=4). Similarly, the rate

Table 1

<table>
<thead>
<tr>
<th>Flow conditions</th>
<th>Constrictor</th>
<th>Pre-circuit EC₅₀ (μM)</th>
<th>Post-circuit EC₅₀ (μM)</th>
<th>Pre-circuit peak tension (g)</th>
<th>Post-circuit peak tension (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous</td>
<td>Phe</td>
<td>10±2</td>
<td>10±2</td>
<td>2.5±0.3</td>
<td>3.0±0.4</td>
</tr>
<tr>
<td></td>
<td>5-HT</td>
<td>1±0.4</td>
<td>1±0.4</td>
<td>4.4±0.5</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>nd</td>
<td>nd</td>
<td>3.9±0.5</td>
<td>4.0±0.9</td>
</tr>
<tr>
<td>Arterial</td>
<td>Phe</td>
<td>14±5</td>
<td>2±1*</td>
<td>4.4±0.7</td>
<td>10.2±1.1**</td>
</tr>
<tr>
<td></td>
<td>5-HT</td>
<td>1±0.4</td>
<td>0.2±0.06*</td>
<td>4.4±0.5</td>
<td>6.7±1.1**</td>
</tr>
<tr>
<td></td>
<td>KCl</td>
<td>22±3×10⁻³</td>
<td>23±4×10⁻³</td>
<td>3.9±0.5</td>
<td>9.8±1.5**</td>
</tr>
</tbody>
</table>

Vein rings were contracted with phenylephrine (Phe), 5-hydroxytryptamine (5-HT) and KCl: all flow conditions were imposed for 90 min. nd, not determined. The application of venous flow for 90 min did not alter the vasomotor responses.

*Significant decrease in EC₅₀, P≤0.05. **Significant increase in peak tension, P≤0.01.
of contraction, in response to 3 μM phenylephrine, increased from 0.11 to 0.37 g/min, \( P<0.02 \) (\( n=4 \)), and spontaneous action potentials were often observed after arterial flow conditions (see example in Ref. 14). These changes were not unique to phenylephrine. After 90 min of arterial flow conditions, heightened sensitivity and contractility to 5-HT was observed (Fig. 1). After saphenous vein was exposed to arterial flow conditions for 90 min the \( \text{EC}_{50} \) for 5-HT decreased from 1.0±0.4 to 0.2±0.06 μM, \( P=0.03 \) and the maximum tension increased from 4.4±0.5 to 6.7±1.1 g, \( P=0.01 \) (\( n=10 \)) (Table 1). In contrast, there was no change in the sensitivity of the vein to KCl after arterial flow conditions for 90 min, although the contractility had increased (Table 1). After vein rings (before and after 90 min arterial flow) had been maximally contracted with 80 mM KCl, further addition of phenylephrine (0.1–100 μM) did not increase the peak tension (\( n=4 \)). The increased peak tension and rate of force generation, in response to agonists, after arterial flow conditions, suggest an increase in the phosphorylation of myosin light chain. However, since phenylephrine did not augment the tension resulting from depolarisation induced increases in calcium (KCl), this is not a classical calcium-sensitising response.

### 3.2. External stenting, vasodilators, kinase inhibitors and Y-27632 attenuate the response to arterial flow

When vein was externally stented with PTFE to limit the circumferential deformation [10], after 90 min of arterial flow conditions, the \( \text{EC}_{50} \) for phenylephrine, 10±2 μM, was similar to that in freshly excised vein \( \text{EC}_{50} \) 17±4 μM, four paired samples, \( P=0.77 \) and the peak tension did not change (Table 2). When the perfusate of unstented veins contained vasodilators (5 μM cromakalim or 10 μM sodium nitroprusside) the heightened sensitivity to phenylephrine and increased contractility of vein rings after arterial flow conditions was abolished (Table 2). In contrast, inclusion of cycloheximide (10 μM) in the perfusate (to block protein synthesis) or gadolinium (to block stretch-activated cation channels) had no such effect (Table 2). After inclusion of gadolinium in the perfusate spontaneous activation always was observed in phenylephrine-induced tension generation. The role of kinases was investigated by the inclusion of kinase inhibitors in the perfusate. Inclusion of H7 (general kinase inhibitor, 1 μM), H89 (PKA inhibitor, 1 μM), or chelerythrine (PKC inhibitor, 1 μM) resulted in flaccid vein rings, which did not contract adequately (<0.8 g) in response to phenylephrine (3×10^{-4} M). At higher concentrations of these inhibitors (10 μM) vein was non-contractile. Genistein

### Table 2

<table>
<thead>
<tr>
<th>Flow conditions</th>
<th>Time (min)</th>
<th>( n )</th>
<th>( \text{EC}_{50} ) (μg)</th>
<th>Peak tension (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>pre</td>
<td>post</td>
</tr>
<tr>
<td>Arterial</td>
<td>90</td>
<td>8</td>
<td>14±5</td>
<td>2±1**</td>
</tr>
<tr>
<td>Arterial+5 μM</td>
<td>90</td>
<td>6</td>
<td>12±4</td>
<td>7±2</td>
</tr>
<tr>
<td>Cromakalim</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial+100 μM</td>
<td>90</td>
<td>4</td>
<td>10±2</td>
<td>2±1**</td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial+10 μM</td>
<td>90</td>
<td>4</td>
<td>10±4</td>
<td>2±0.7*</td>
</tr>
<tr>
<td>Gd³⁺</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial+10 μM</td>
<td>90</td>
<td>4</td>
<td>11±2</td>
<td>2±0.6*</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial+PTFE</td>
<td>90</td>
<td>4</td>
<td>17±4</td>
<td>10±2</td>
</tr>
<tr>
<td>Stent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial+Y-27632</td>
<td>90</td>
<td>5</td>
<td>1.3±4</td>
<td>1.4±5</td>
</tr>
</tbody>
</table>

Results are reported for freshly excised tissue (pre) and tissue after exposure to arterial flow conditions for 90 min. nd, not determined, peak tension was that observed at a phenylephrine concentration of 300 μM.

*\( P<0.05 \); ** \( P<0.005 \) paired t-test.
that circumferential deformation stimulates Rho kinase-dependent signalling pathways, which lead to the increased contractility and sensitivity to phenylephrine of saphenous vein exposed to arterial flow conditions.

3.3. Regulation of myosin light chain phosphorylation

The contractile force generated by smooth muscle depends on the extent of myosin light chain phosphorylation, which is regulated positively by myosin light chain kinase (MLCK) and negatively by a Rho kinase-dependent phosphatase. Western blotting and slot blotting experiments, respectively, indicated that the concentration of MLCK and the ratio of MLCK/GAPDH increased 2-fold after 90 min of arterial flow conditions (Fig. 3). Attempts to quantify the amount of phosphorylated myosin light chain by combined gel electrophoresis and antibody techniques were unsuccessful, since the enhanced chemiluminescence reagents appeared to bind non-specifically to components of the medial smooth muscle lysates.

4. Discussion

Human saphenous vein has unique properties, which allow it to respond to rapid changes in pressure, 80 mmHg or more, when a person moves from a recumbent to a standing position. The myogenic reflex and valves are examples of a physiological mechanism and anatomical
structure that facilitates this adaptation and prevents venous blood pooling in the foot on standing. Saphenous vein also can adapt to more extreme changes in haemodynamic force, when it is used as a conduit for arterial bypass. Here we show, that in the absence of external stenting, exposure of saphenous vein to arterial flow conditions for up to 90 min, was associated with a 2–3-fold increase in the maximum tension sustained by vein rings and an increase, by an order of magnitude, in sensitivity to vasoconstrictors.

The change in peak tension and the altered sensitivity to vasoconstrictors appears to depend on Rho kinase signalling pathways. Both changes appear to arise from circumferential deformation of the vein, since they can be reversed by external stenting.

Freshly excised saphenous vein rings, or veins exposed to simulated venous flow for up to 90 min, exhibit a slow rate of contraction in response to phenylephrine or 5-HT: this was independent of the presence of endothelium. These agonists did not cause a further increase in tension of vein rings depolarised with KCl. Therefore, saphenous vein smooth muscle appears to contract via a latch mechanism using dephosphorylated cross-bridges (characteristic of tonic smooth muscle) and does not appear to exhibit classic calcium-sensitisation [15–17]. This suggests that normal contraction of saphenous vein does not depend on large increases in the phosphorylation of myosin light chain.

Following exposure of vein to simulated arterial flow, the contractile properties of the smooth muscle alter rapidly. The muscle can generate increased tension, generate tension more rapidly and may develop spontaneous action potentials [14]. The first two of these changes indicate that generation of tension is now a result of large increases in myosin light chain phosphorylation [16,17]. This could result from either increased activity of MLCK or decreased activity of myosin light chain phosphatase (SMPP1M). It is a weakness of our study that we were unable to quantify myosin light chain phosphorylation directly. However, perfusion of vein with the selective Rho kinase inhibitor Y-27632 abolished the increased rate and magnitude of tension generation, observed after arterial flow conditions. Recently, the specificity of Y-27632 has been questioned, indicating that Y27532 also may inhibit some other kinases [18]. Saphenous veins are thick and we used a relatively high concentration of Y-27632 (20 μM), but other reports indicate that selective inhibition of Rho kinase is maintained at these concentrations [19,20]. Therefore, activation of Rho kinase is likely to be the principal mechanism underlying the rapid changes (within 5 min) in peak tension of saphenous vein, in response to arterial flow conditions. The apparent increase in MLCK after 90 min of arterial flow conditions may have some synergistic effect. Indeed, increased contractility of sensitised canine saphenous vein has been attributed to the elevation of MLCK content in the smooth muscle [21]. Further, in isolated airway smooth muscle cells mechanical strain increased the magnitude and velocity of cell shortening, associated with an increasing content of MLCK [22]. Inclusion of 10 μM cycloheximide in the vein perfusate did not abolish the heightened sensitivity, in response to phenylephrine, of human saphenous vein rings after 90 min arterial flow (Table 2). Therefore we can infer that, if the increase in total MLCK content contributes to the sensitisation of human saphenous vein medial smooth muscle, the increase in MLCK content must have arisen from reduced MLCK degradation. In saphenous vein, activation of Rho kinase signalling appears to result from circumferential stretching of the smooth muscle, rather than increased pressure or shear stress, since the effects on agonist-induced peak tension were abrogated by external stenting and vasorelaxants (cromakalim and sodium nitroprusside). Although the smooth muscle cells are stretched by ~30% during each flow cycle [12], the inclusion in the vein perfusate of gadolinium to block stretch-activated ion channels, also failed to abolish the response. Stretch of localised areas of the smooth muscle cell membrane, in series with both the Rho kinase signalling pathway and the contractile apparatus, may trigger both the altered mechanism of muscle contraction and the development of spontaneous action potentials. Similar Rho kinase-dependent mechanisms have been described as leading to interleukin-1 induced coronary artery contraction and spasm in swine [19]. These authors focused on 5-HT-induced contraction and did not report whether the EC₅₀ altered.

In saphenous vein, although the EC₅₀ for KCl (electromechanical coupling) was unchanged after arterial flow conditions, the EC₅₀ for both phenylephrine and 5-HT (pharmacomechanical coupling) had decreased by almost an order of magnitude. This change in pharmacomechanical coupling was abrogated by external stenting of the vein, perfusion with the Rho kinase inhibitor Y-27632 or a vasorelaxant (cromakalim). Previously, signalling via protein kinase C and small GTP-binding proteins, rather than Rho kinase signalling, has been considered to contribute to the sensitisation process [23,24]. However, in saphenous vein classic calcium sensitisation was not apparent and our results indicate an important role for Rho kinase in the altered pharmacomechanical coupling evident after arterial flow conditions. Such changes in pharmacomechanical coupling could aggravate vasospasm in newly implanted vein grafts.

In 1984 Seidel et al. reported that canine femoropopliteal saphenous vein grafts developed an elevated sensitivity to norepinephrine 1–8 weeks after implantation [25]. A heightened sensitivity to phenylephrine has been reported when human saphenous vein has been subjected to pulsatile flow at arterial pressures ex vivo [2,12]. When aortocoronary or femoropopliteal vein grafts have failed, the vasomotor responses of arterialised vein have been investigated, although little data are available until 8–12 months after implantation. At this time the sensitivity of
References


Acknowledgements

This work was supported by the Graham Dixon Trust, the Peel Medical Research Trust and the British Heart Foundation. We appreciate the co-operation of Professors R.M. Greenhalgh, and K.M. Taylor in providing saphenous vein.
