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The Effects of Potassium Channel Openers on Saphenous Vein Exposed to Arterial Flow*

D. K. Beattie†, M. Gosling, A. H. Davies and J. T. Powell†

Departments of Surgery, Charing Cross & Westminster Medical School, St Dunstan's Road, London W6 8RP

Objectives: *To assess the sensitivity of saphenous vein to potassium channel opening drugs (KCOs).*
Methods: *Saphenous vein, harvested at bypass surgery or high ligation for correction of varicose veins, was exposed to an* in vitro *flow circuit and vasomotor responses assessed by organ bath pharmacology.*
Outcome measures: *Effective drug concentrations for 50% reduction in vein ring tension (IC_{50}).*
Results: *Vein rings pre-contracted with phenylephrine showed a concentration-dependent relaxation to all the KCOs tested with a potency ranking of HOE 234>cromakalim>pinacidil>diazoxide. The relaxation to cromakalim was endothelium-independent and was inhibited by glibenclamide (an ATP-sensitive K^+ channel blocker). The sensitivity of vein rings to cromakalim increased after exposure to arterial flow conditions for 90 minutes (IC_{50} before 1.7 ± 0.25 μM and after 0.25 ± 0.08 μM, p >0.001). This effect was not evident after 90 min of venous flow conditions, 2.19 ± 0.49 μM. When the workload on vein, exposed to arterial flow conditions, was reduced mechanically by external stenting with PTFE the increased sensitivity to cromakalim was abolished.*
Conclusion: *Saphenous vein has ATP-sensitive K^+ channels responsive to KCOs. The increased sensitivity to cromakalim, induced by arterial flow conditions, may represent an endogenous protective mechanism limiting ischaemic damage resulting from the higher workload imposed on grafted vein.*

Key words: Saphenous vein; Bypass; K_{ATP}; Channel; Cromakalim.

Introduction

Human saphenous vein remains the most widely used conduit in peripheral arterial reconstruction. Nevertheless up to 30% of grafts fail, usually within 1 year.[1] Trauma, hypoxia and the exposure of the vein to the haemodynamics of arterial flow are all thought to contribute to the tissue remodelling which occurs.[2] The structural changes in the vein include the development of intimal hyperplasia secondary to smooth muscle cell replication and migration.[3] Functional changes include increased catecholamine sensitivity[4,5] and alterations in endothelium-dependent vasorelaxation.[6] The increased workload to which the vein is subjected in the arterial circulation probably is pivotal to the adaptive response and related structural and functional changes.

Potassium channel opening drugs (KCOs) are a relatively new class of drug whose vasorelaxant properties already are being exploited to reduce workload in angina, hypertension and in peripheral vascular disease.[7–10] The mechanism of action of KCOs is attributed to activation of ATP-dependent potassium (K_{ATP}) channels. K_{ATP} channels were first described in smooth muscle in 1989.[11] Their function is related to intracellular ATP concentration; reduction of ATP as a result of metabolic stress leads to K_{ATP} channel activation, reduced cell excitability, and hence a reduction in workload. The K_{ATP} channel is regulated directly by the concentrations of intracellular ATP. As ATP falls, K_{ATP} channels become active, resulting in K^+ efflux from cells and cellular hyperpolarization. These changes oppose the opening of Ca^{2+}-permeable channels and promote vasorelaxation. Thus K_{ATP} channels represent an elegant physiological mechanism through which the contractile ability of a muscle cell is directly coupled to its metabolic status. The presence of K_{ATP} channels in saphenous vein has been suggested, since the potassium channel opener levcromakalim relaxes precontracted vein.[12] The suggested clinical uses of KCOs include ischaemic pre-conditioning.[13] In this study we have investigated the hypothesis that

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† Please address all correspondence to: D. K. Beattie or J. T. Powell.

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saphenous vein smooth muscle cells have K_{ATP} channels, which could be manipulated pharmacologically with KCOs to provide ischaemic pre-conditioning of venous bypass grafts.

Material and Methods

Vein harvesting and perfusion

Segments of human saphenous vein were taken from non-diabetic patients undergoing bypass surgery, both peripheral and cardiac, amputation and high ligation for correction of varicose veins. The use of saphenous vein, for the investigations reported here, was approved by the local Ethical Committee. Veins were transported immediately to the laboratory in fresh, cooled pre-oxygenated Kreb's solution (4 °C, 95% O_2, 5% CO_2). Utilised vein was non-distended and had not been perfused. All loose connective tissue was removed from the vein and a ring of vein taken as a control. A further segment of vein was removed, immediately snap-frozen in liquid nitrogen, and stored at −70 °C for later ATP analysis. The tissue was weighed before being pulverised in liquid nitrogen and extracted into 0.9 M perchloric acid. ATP was quantified by an enzymatic method.[14]

The remaining segment of vein was mounted on a retaining jig and placed into an *in vitro* circuit as described previously.[15] Briefly, this consisted of a litre perfusate reservoir connected to the retaining jig via a cardiac bypass pump (Stockert Instrumente, Munich), thus allowing variations in flow rate, pressure and frequency to be made. Vein segments were perfused with warmed, oxygenated Kreb's solution (37 °C, 95% O_2, 5% CO_2) and the retaining jig was immersed in a similar solution to externally bathe the vein. Modified Kreb's solution (NaCl 118.4, KCl 4.7 mmol, KH_2PO_4 1.2 mmol, $MgSO_4 \cdot 7H_2O$ 1.2 mmol, glucose 11.1 mmol, $NaHCO_3$ 24.9 mmol, $CaCl_2$ 2.5 mmol) was made freshly each day. Flow rate was measured at the beginning and end of each period of perfusion and the perfusion was continually monitored. Perfusate temperature was measured at two points in the circuit. The diameters of the vein wall during perfusion was recorded using a combination of B and M mode ultrasound (Aloka SSD500) with 7.5 MHz linear transducer.

Vein segments were perfused under the following conditons:

Arterial flow, in a pulsatile manner at 90 cycles per minute, with a mean pressure of 100 mmHg and flow rate of 200 ml/min for 90 min.

Venous flow, in a non-pulsatile manner with a pressure of 20 mmHg and a flow rate of 10 ml/min for 90 min.

Organ Bath Experiments

Vein rings were mounted on 0.2 mm stainless steel stirrups placed through the lumen in organ baths of 10 ml internal capacity. One stirrup was fixed whilst the other was connected to an isometric force transducer (Harvard Apparatus, Massachusetts) and changes in tension generated by the rings recorded in both electronic (MacLab Software®) format and on hard copy (Student Oscillograph, Harvard Apparatus, Kent). The organ bath was filled with warmed oxygenated Kreb's solution (37 °C, 95% O_2, 5% CO_2). Vein rings were initially placed under 2 g of tension and allowed to relax to equilibrium.

Vein rings were contracted with potassium chloride (40 mM), washed out and recontracted, submaximally, with phenylephrine. The relaxation of vein rings in response to increasing concentrations of the potassium channel openers cromakalim (Sigma, UK), HOE 234 (a gift from Hoechst, Germany), pinacidil and diazoxide. For some experiments vein rings were pre-incubated with glibenclamide (Sigma, UK). The presence of functional endothelium on vein rings was assessed from relaxation of pre-contracted vein rings to the calcium ionophore, A23187. Vein rings were denuded of endothelium by passing a wire across the lumenal surface. Stock solutions of diazoxide, pinacidil and HOE 234 were made up in DMSO (Sigma, UK) whilst cromakalim and glibenclamide were dissolved in ethanol (British Drug Houses, UK). Vehicle controls using the maximum concentration of these solvents caused no relaxation of vein rings precontracted with phenylephrine.

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 (EC_{50}) for phenylephrine were calculated by normalising data to the peak response and fitting the concentration-response curves to a logistic plot incorporating Hill coefficient using MicroCal Origin (MicroCal Inc., Northampton, MA, USA). The concentrations of potassium channel openers causing 50% inhibition of contraction (IC_{50}) were calculated in a similar manner. 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).

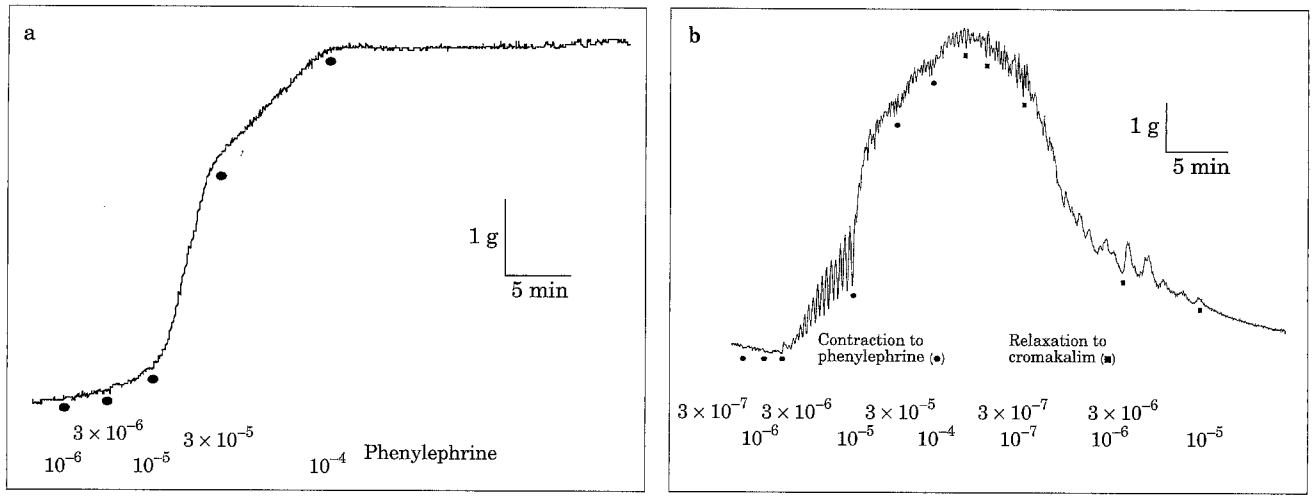

Fig. 1. (a) Oscillograph tracing to show the maintained contraction of a freshly excised saphenous vein ring precontracted with phenylephrine up to 0.1 mM. (b) Oscillograph tracing to show the relaxation of a freshly excised saphenous vein ring, precontracted with phenylephrine (0.1 mM), to increasing concentrations of cromakalim.

Results

Functional, non-diseased vein supported a contraction of >2 g (mean 4.4±0.7 g) in response to increasing concentrations of phenylephrine, EC_{50} 10.9±2.0 μM. The ATP content of this freshly excised vein was 385±81 nmol/g tissue wet weight. After vein has been exposed to either venous or arterial flow conditions for 90 minutes in the *in vitro* circuit, the ATP concentration remained unchanged. After venous circuits the EC_{50} for phenylephrine (9.2±1.2 μM) was unchanged, but after arterial circuits the EC_{50} for phenylephrine had decreased to 2.1±0.5 μM, p <0.001 compared with freshly excised vein.

After vein rings had been submaximally contracted with phenylephrine, increasing concentrations of potassium channel openers (KCOs) effected increasing vasorelaxation. The response to cromakalim is shown in Figure 1. The concentration-dependence of this relaxation was similar in veins with endothelium and vein rings denuded of endothelium (Figure 2). The concentration of cromakalim which caused 50% relaxation (IC_{50}) was 1.7±0.25 μM. Pre-incubation of the vein ring with 30 μM glibenclamide (a K_{ATP} channel blocker) abolished the relaxation in response to cromakalim (Figure 2).

The potency of four different KCOs in causing relaxation of pre-contracted vein rings was compared. The most potent drug was HOE 234 (IC_{50} 0.2±0.04 μM), followed by cromakalim (IC_{50} 1.7±0.25 μM), and pinacidil (IC_{50} 5.8±0.7 μM), with diazoxide being the least potent (IC_{50} 36±4.4 μM), see Table 1. Cromakalim was used for all further experiments.

When vein is exposed to arterial flow conditions *in vitro*, there is pronounced cyclical distension of the

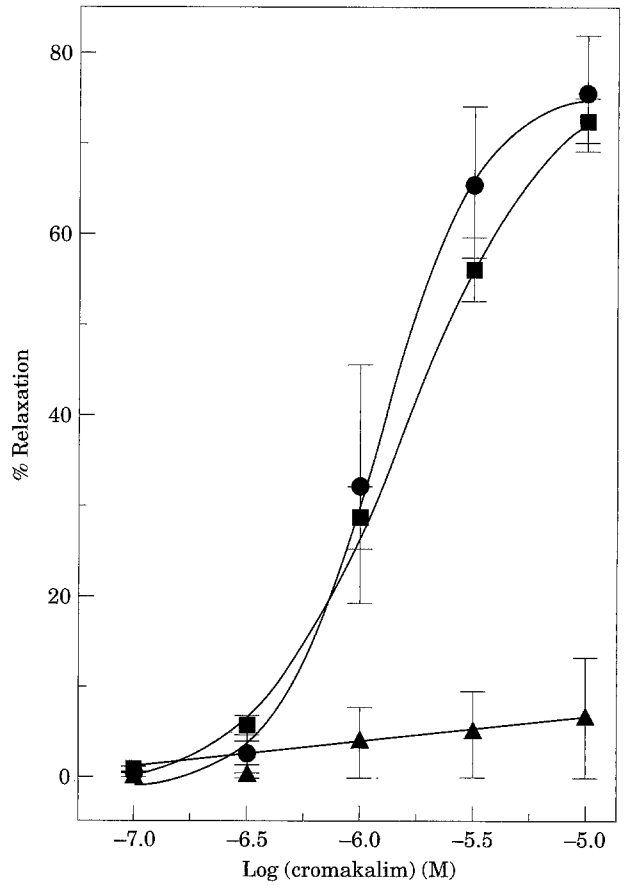

Fig. 2. The response to cromakalim is endothelium-independent and blocked by glibenclamide. The relaxation of pre-contracted vein rings, with endothelium (●) or denuded of endothelium (■), in response to increasing concentrations of cromakalim. The relaxation was abolished when vein rings were pre-incubated with 100 μM glibenclamide (▲).

Table 1. The sensitivity of human saphenous vein to the vasorelaxant effect of potassium channel opening drugs.

	Diazoxide	Pinacidil	Cromakalim	HOE234
IC_{50} for freshly isolated vein μM (S.E.M.)	38±1.3	3.16±1.25	1.7±0.25	0.043±0.012
IC_{50} for vein after 90 min of arterial flow μM (S.E.M.)	n.d.	1.70±0.77	0.25±0.08*	n.d.

n.d. = not determined.
* Significant reduction compared to freshly excised vein, p <0.0001.

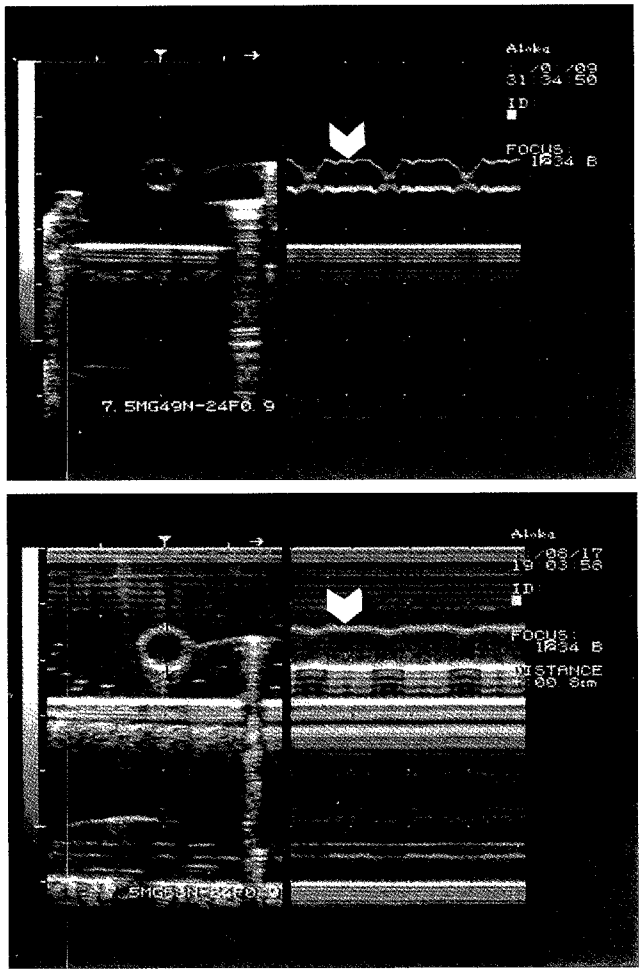

Fig. 3. Pulsatile circumferential distension of vein exposed to arterial flow is limited by external stenting. The B mode image is shown on the left-hand side of each picture. On the right the M-mode trace follows wall motion through three simulated arterial pulse cycles: (a) the large change in diameter of unstented vein (arrow); (b) the limited change in diameter of stented vein (arrow).

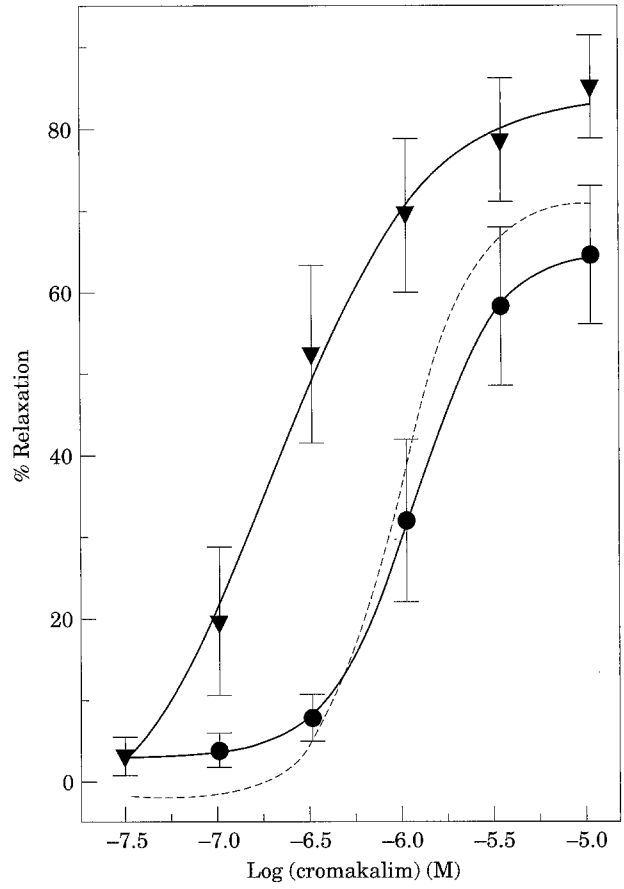

Fig. 4. The heightened sensivity to cromakalim of vein exposed to arterial flow conditions is abolished by external stenting. The relaxation of pre-contracted vein rings to cromakalim after exposure to venous flow conditions for 90 min (●) or arterial flow conditions for 90 min (▼). These latter vein rings relax at much lower concentrations of cromakalim. The interrupted line shows the response of stented veins exposed to arterial flow for 90 min; the sensitivity to cromakalim has not increased.

vein, with peak venous diameter increasing by −35% with each cycle.[16] This change in diameter can be observed by the M-mode ultrasonography (Figure 3a). This cyclical circumferential deformation can be limited by placing the vein in an external PTFE stent (Figure 3b). This limitation of external stenting is likely to decrease mechanically the workload on the vein exposed to arterial flow.

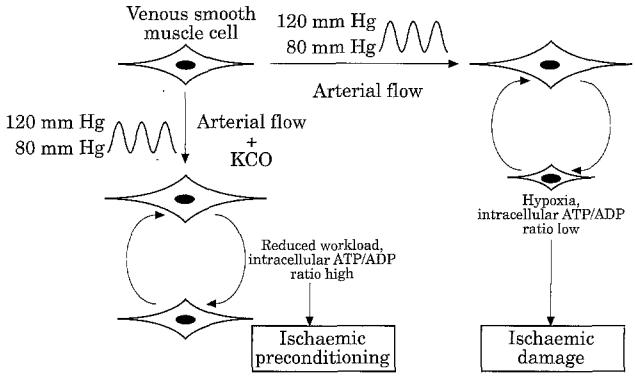

Fig. 5. Hypothesis for mechanism of vein graft protection by potassium channel openers (KCOs).

After vein had been exposed to arterial (but not venous) flow conditions for 90 min the IC_{50} for cromakalim decreased significantly from 1.7 ± 0.25 μM to 0.25 ± 0.08 μM, $p < 0.001$ (Figure 4). After venous flow conditions for 90 min the IC_{50} for cromakalim was 2.19 ± 0.49 μM. This increased sensitivity of vein to cromakalim was observed only in unstented vein: the IC_{50} for cromakalim (1.02 ± 0.19 μM) remained unchanged in stented vein (Figure 4).

Since external stenting of vein exposed to arterial flow for 90 min abolished the increased sensitivity of vein rings to both phenylephrine[16] and cromakalim, these changes appear to be a response to increased workload. Therefore, we considered whether pre-conditioning vein by inclusion of cromakalim in the vein perfusate could decrease the workload pharmacologically and abolish the increased sensitivity of arterial-perfused vein rings to phenylephrine. When 5 μM cromakalim was included in the Kreb's solution perfusing veins exposed to arterial flow for 90 min the contractile response to phenylephrine was normalised, the EC_{50} increasing from 2.1 ± 0.5 μM in the absence of drug to 7.0 ± 2.2 μM in the presence of cromakalim.

Discussion

Here we report that human saphenous vein smooth muscle cells possess ATP-sensitive K^+ channels which are sensitive to potassium channel opening drugs. Potassium channel openers are finding increasing clinical usage for cardiovascular disease, particularly in the area of ischaemic pre-conditioning.[13] When saphenous vein is used as an arterial bypass conduit the workload increases as a result of the pulsatile arterial flow and the increased sensitivity of the newly implanted vein bypass to circulating catecholamines acts in synergy to increase the graft workload.[14,16,17] This combination is predicted to promote muscle hypoxia and decrease intracellular ATP/ADP ratio (Figure 5). Such muscle hypoxia has been suggested to promote the development of intimal hyperplasia.[18] The relaxant and metabolic effects of potassium channel openers could be used to avert these unwanted effects: this is termed clinically as ischaemic preconditioning.

The potassium channel openers currently approved for clinical use in Britain include nicorandil, diazoxide and minoxidil. Other drugs, including pinacidil, are approved in Europe and many more are under development. The drugs used in this study belong to three different structural classes: benzopyrans (e.g. cromakalim, HOE 234), cyanoguanidines (pinacidil) and benzothiadiazones (diazoxide), and we have shown that, for saphenous vein, the benzopyrans are the most potent of the potassium channel openers. The effect of benzopyrans to relax pre-contracted vein rings was independent of the presence of endothelium but could be inhibited by the oral hypoglycaemic drug glibenclamide.

To enable investigation of the adaptation of saphenous vein to arterial flow, we have developed and validated an *in vitro* flow circuit.[15] After exposure of saphenous vein to arterial flow conditions for 90 min the sensitivity of vein rings to cromakalim was increased significantly, the IC_{50} decreasing from 1.7 μM in freshly excised vein to 0.25 μM after arterial flow. In contrast, the sensitivity of veins to the less potent KCO, pinacidil remained unchanged. The increased sensitivity to cromakalim was not observed when veins were exposed to venous flow *in vitro*. These data imply that ATP-sensitive potassium channels participate in the adaptive responses of newly implanted saphenous vein grafts.

External stenting of saphenous vein with PTFE attenuates the workload on new vein grafts. Experimentally the external stenting of porcine interposition vein grafts has been demonstrated to reduce intimal thickening and maintain lumen diameter.[19] Interestingly, snug-fit or over-sizing of the external porous stent did not alter these beneficial effects. External stenting also has been shown to attenuate some of the endothelial changes which occur when saphenous vein is exposed to arterial flow.[16] Following these results some cardiac surgeons have initiated a clinical trial of external stenting of both aortocoronary and femorodistal vein grafts. Our results indicate that KCO therapy could provide an alternative approach to reduce the workload on newly inplanted vein grafts.

In our *in vitro* studies, external stenting of the saphenous vein abolished the arterial flow-induced increase in sensitivity to cromakalim observed in unstented vein. The ultrasonographic studies confirm that the circumferential distension of saphenous vein

is limited by external stenting (Figure 3).[16] In the presence of constant flow, if circumferential deformation is limited shear stress increases: in saphenous vein exposed to arterial flow this could increase the shear stress in externally stented veins by ~50%. This increase in shear stress may have separate effects, transduced by the endothelium, on the underlying smooth muscle. Therefore, the increased sensitivity to cromakalim, commented on above, which is endothelium-independent and abolished in externally stented vein, is a consequence of circumferential deformation and not increased shear stress.

The practice of ischaemic pre-conditioning may be a novel approach for the vascular surgeon. There are reports that potassium channel openers are in clinical trial for the treatment of intermittent claudication.[13] We are reluctant to pursue our studies in experimental models, since although many drugs have proved effective in limiting intimal hyperplasia in animal models none have been effective in man. Therefore, our current approach is to extend our *in vitro* studies before suggesting appropriate drugs for clinical evaluation.

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