Endothelium-dependent Responses in Autogenous Femoral Veins Grafted into the Arterial Circulation of the Dog

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Abstract

Endothelium-dependent responses differ in arteries and veins of the dog. Experiments were performed to determine whether chronic grafting of veins into the arterial circulation would alter the endothelium-dependent responses of the veins. Segments of femoral veins were grafted to the femoral artery of the dog. 6 wk after surgery the venous grafts were removed from the dog, cut into rings, and suspended in organ chambers for isometric tension recording. In some rings the endothelial cells were removed. Acetylcholine and α_2-adrenergic agonists did not cause endothelium-dependent relaxations in venous grafts. The calcium ionophore (A23187) initiated such relaxations which were not mediated by prostanoids. Endothelium-dependent relaxations were also observed in venous grafts to ADP, thrombin, and arachidonic acid. In segments of graft where *myo*-intimal hyperplasia was prominent, relaxations to ADP, thrombin, and A23187 were blunted and in some segments contractions were observed. These results demonstrate the ability of the endothelium of venous grafts to initiate changes in tone of the smooth muscle.

Introduction

The endothelial cells can modify the smooth muscle reactivity of arteries and veins of the coronary, pulmonary, and systemic vasculature (1, 2). The responses initiated by the endothelial cells can be modified by chronic changes in blood flow and oxygen tension (3). The role of the endothelium in initiating and modifying the tone of veins grafted into the arterial circulation is unknown. As venous grafts are exposed chronically to elevated blood flow, pressure, and oxygen tension, the present experiments were designed to determine whether endothelium-dependent responses occur in venous grafts and, if so, to determine how these responses differ from those observed in unoperated veins.

Methods

Surgical procedures. Male mongrel dogs (20–30 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). During the operative procedure the animals received 100 U/kg heparin sulfate and were volume expanded with 1 liter Plasma-lyte (meq/liter: 140, sodium; 5, potassium; 3, magnesium; 98, chloride; 27, acetate; 23, gluconate) intravenously. Each animal received antibiotics (5 mg/kg gentamicin sulfate; 60,000 U/kg penicillin G preoperatively). Using aseptic surgical techniques, the skin over the femoral triangle of the left leg was opened and the caudal portion of the sartorius muscle retracted. The femoral artery and vein were exposed for a 10 cm length, and smaller branches and tributaries were ligated flush with the vessels and divided. The femoral artery was clamped proximally and distally with DeBakey clamps and divided distally. The femoral vein was doubly ligated distally, divided between the ligatures, ligated proximally, and excised. The vein emptied of blood by gravity flow through the proximal end. This proximal segment of vein was immediately reversed and anastomosed to the distal limb of femoral artery without prior flushing or distension, using a spatulated end-to-end anastomosis with 7-0 running prolene. The vein was then placed on gentle traction using the previously placed ligature and cut, with the proximal femoral artery stump, to the appropriate length. A spatulated end-to-end anastomosis was performed proximally, again with 7-0 running prolene. Before completion of the proximal anastomosis, the graft was filled with blood retrograde and prograde and the DeBakey clamps removed. Care was taken throughout the procedure to avoid clamping or other instrumentation of the vein segment. The time from vein excision to completion of the anastomosis and establishment of blood flow was not longer than 15 min. The incision was then closed with running 2-0 vicryl in the subcutaneous layer and running 3-0 vicryl in the cutaneous layer. The blood vessels from the right limb served as control tissues. Upon recovery from anesthesia, none of the animals exhibited impairment of mobility of the hind limbs or infection.

In vitro experiments. After a 6-wk period, the animals were anesthetized again with sodium pentobarbital. The femoral artery and vein of each limb were exposed. The gracilis branch of the artery from the unoperated limb was cannulated for the monitoring of blood pressure (P23 pressure transducer, Statham, Hato Rey, PR; model 1108 Visicorder [Honeywell Inc., Pleasantville, NY]). Blood flow was measured through the unoperated artery and the artery proximal to the venous graft (electromagnetic flow probe and meter [Carolina Medical Electronics, Inc., King, NC]). The dogs then were exsanguinated via the carotid artery and the unoperated femoral vein and venous graft at the level of the arterial anastomoses were removed. The excised vessels were placed in a chilled modified Krebs-Ringer bicarbonate solution (buffered salt solution) of the following millimolar composition: NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; CaEDTA, 0.026; glucose, 11.1.

After the blood vessels were cleaned of connective tissue with care not to touch the luminal surface, rings 5 mm in length were cut. In some rings the endothelium was removed by rubbing the luminal surface gently with a cotton swab wetted with the buffered salt solution (4). The rings were suspended in an organ chamber between a clip and a force transducer (UTC-2, Gould Inc., Cleveland, OH) by two stainless steel wires inserted into the lumen of the vessel. The organ chamber was filled with 25 ml of buffered salt solution at 37°C and gassed with 95% O_2–5% CO_2. Changes in isometric force were measured. Each ring was stretched to the optimal point of its length-tension curve (L_0) as determined by the maximal tension developed to 3×10^{-7} M norepinephrine (Table I). After a 30-min equilibration period, a cumulative concentration-response curve to norepinephrine (from

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Table I. Measure of Range of Myo-intimal Thickening in Femoral Venous Grafts

Degree of thickening	Region of graft		
	Proximal	Middle	Distal
	mm	*mm*	*mm*
Least	0.08±0.02*	0.15±0.03	0.21±0.07
Maximal	0.32±0.06	0.35±0.06	0.47±0.08
Average of four quadrants	0.18±0.04	0.25±0.04	0.31±0.08

* Data are expressed as means±SEM; $n = 6$ in all groups.

10^{-8} to 10^{-4} M) was obtained. To study endothelium-dependent relaxations of the blood vessels, each was contracted with either the individual dose of norepinephrine causing 30% of maximal contraction (ED_{30}) or when α_2-adrenergic responses were studied with prostaglandin $F_{2\alpha}$ (2×10^{-6} M). Two protocols were followed.

(*a*) In one set of experiments, one ring of venous graft was cut 0.5–1 cm from each of the proximal and distal anastomoses of the graft and two rings were cut from the middle of the graft (Fig. 1). The endothelium was removed from one of the middle rings of the graft. These four rings of graft were studied in parallel with two rings of unoperated femoral vein (one with and one without endothelium). The endothelium-dependent responses were studied in the following order: acetylcholine, ADP, UK 14,304 (an α_2-adrenergic agonist; reference 4), arachidonic acid, thrombin, and the calcium ionophore A23187. Increasing concentrations of agonist were added cumulatively to the organ bath except for thrombin, which was given as a single dose. The tissues were washed at least three times with buffered salt solution, and at least 30 min elapsed before testing the next drug. A maximal contraction to potassium chloride (60 mM) was obtained at the end of the experiment.

(*b*) In the other set of experiments, two rings were cut sequentially 0.5–1 cm from the proximal anastomoses of the venous grafts, and two rings were cut sequentially from the middle of the venous grafts. The endothelium was removed from the two middle rings. These four rings of venous graft were studied in parallel with four rings of unoperated vein, two with and two without endothelium. A pair of rings with and without endothelium from both the venous graft and unoperated vein were incubated with indomethacin (10^{-5} M for 30 min) to inhibit the production of prostanoids. Cumulative concentration response curves to acetylcholine, ADP, and the calcium ionophore A23187 were obtained in blood vessels contracted with the ED_{30} of norepinephrine.

Drugs. The following drugs were used: acetycholine chloride, ADP, arachidonic acid sodium salt, calcium ionophore A23187, dimethyl sulfoxide (DMSO), indomethacin, l-norepinephrine bitartrate, prostaglandin $F_{2\alpha}$, and bovine thrombin, all from Sigma Chemical Co., St. Louis, MO, and UK-14,304 Tartrate (5-bromo-6[2-imidazolin-2-yl-amino]-quinoxaline, D[+] Tartrate; Pfizer Central Research, Sandwich, England).

The calcium ionophore was dissolved in DMSO (final bath concentration, 8.2×10^{-3} M) and diluted with distilled water; the tissues did not respond to DMSO alone. Indomethacin was dissolved in a solution of Na_2CO_3 (final bath concentration, 2×10^{-5} M). All other

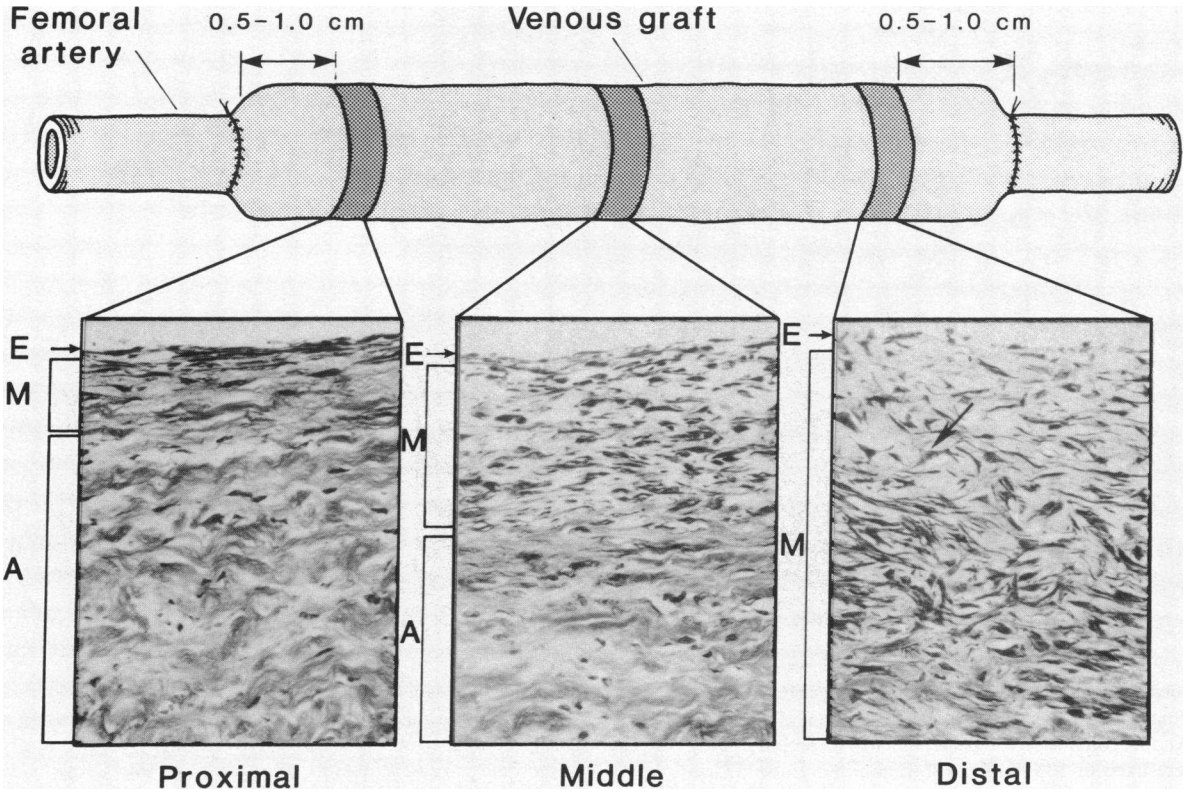

Figure 1. Schematic of the location of rings taken from the venous grafts accompanied by representative histological sections at each location (400×; Masson trichrome stain). In some experiments, two rings were cut from the middle section and endothelial cells were removed from one of these rings (not shown). Symbols represent endothelial layer (*E*), media (*M*), adventia (*A*). In some grafts there was a progressive thickening of the intimal-medial structures from proximal to distal ends. *myo*-intimal thickening was characterized by the presence of stellate myofibroblasts (*arrow*, distal segment).

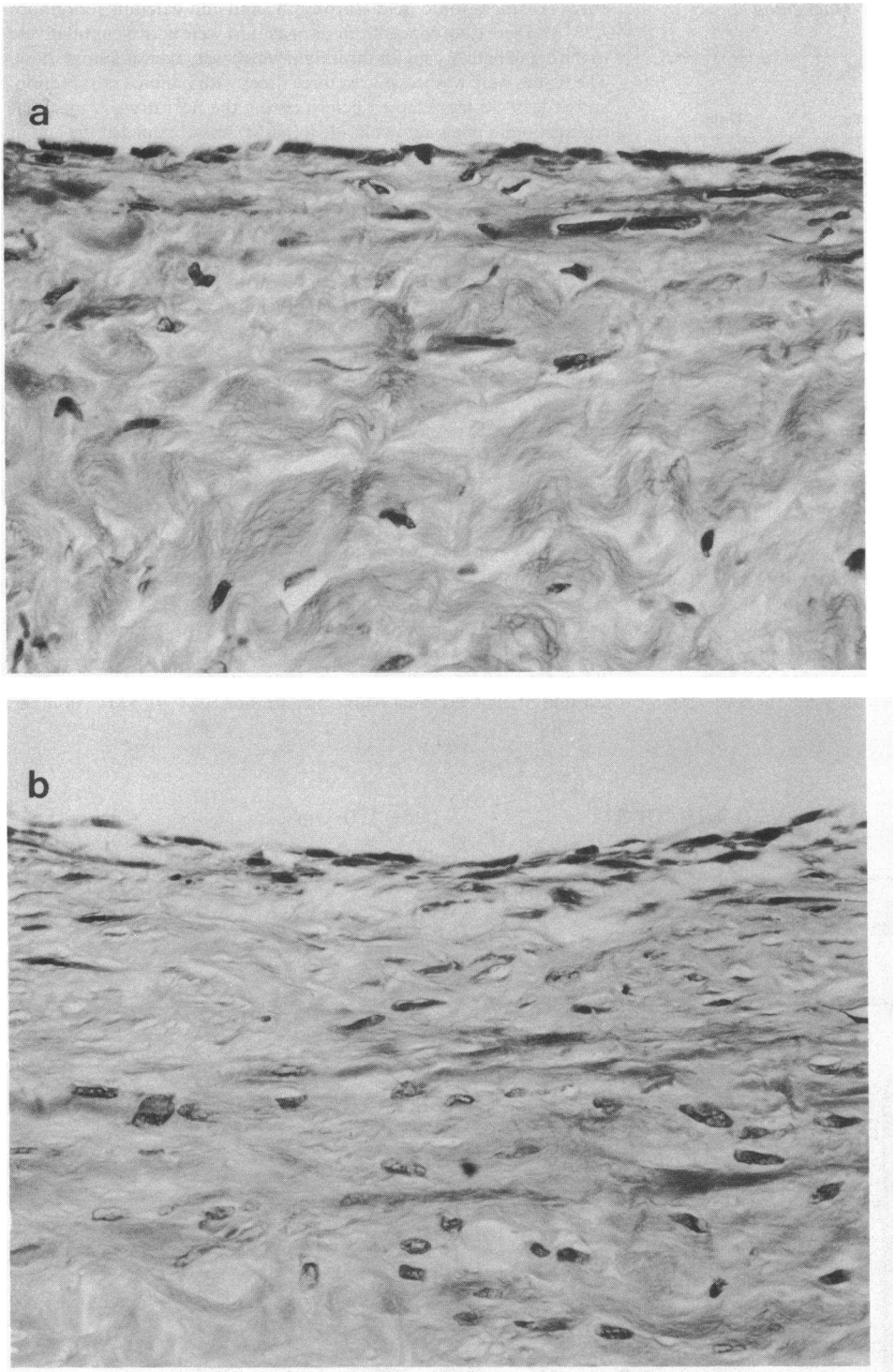

Figure 2. Histological sections (H and E stain) of the sections ([*A*], proximal, [*B*] middle, [*C*] distal) graft shown in Fig. 1 using oil immersion (640×) to identify endothelial cells lining the lumen of the graft.

drugs were dissolved in distilled water, and the concentrations are reported as the final molar (M) concentration in the organ bath.

Histology. At the conclusion of some in vitro experiments the blood vessels were fixed in 10% buffered formalin and embedded in paraffin. Transverse sections (6 μm thick) were cut and stained with either hematoxylin and eosin (H and E) or Masson trichrome stain to examine endothelium and to differentiate smooth muscle from connective tissue, respectively, by light microscopy. Measurement of myointimal thickening was obtained from Masson trichrome-stained sections of proximal, middle, and distal grafts. A single section from each area of graft was assessed for the point of least and maximal distance from the luminal surface to the adventitial boarder using a calibrated grid on a light microscope. Each section was arbitrarily divided into quarters and a measure of *myo*-intimal thickening made at each quarter. These four measures were then averaged to determine the degree of thickening in each section (Table I).

Statistical analysis. 15 dogs were prepared with venous grafts. Of these two were excluded from further study as the grafts were occluded. In all experiments, n = number of dogs. The results are expressed as means±SEM. When rings from a single dog were studied in parallel,

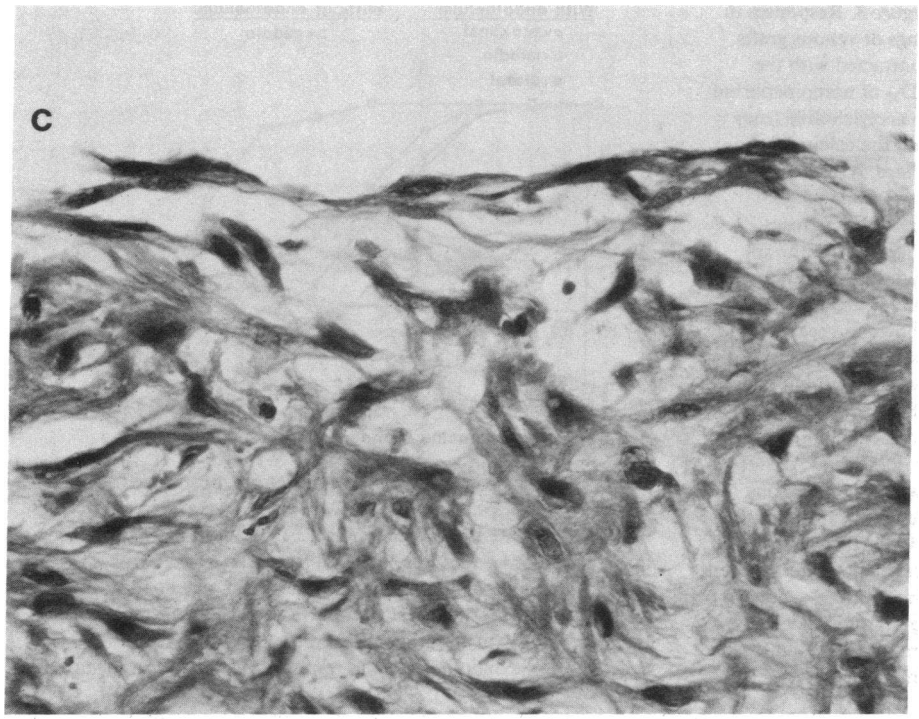

Figure 2 (Continued)

Student's *t* test for paired observations was used. If two or more means were compared, an analysis of variance for one-way classification was used. When a statistically significant F value was found, means were tested using Scheffe's test for multiple comparisons. Where appropriate, the effective dose for 50% of the maximal response (ED_{50}) was calculated for individual concentration-response curves and the mean of these values is reported as the negative log of the molar concentration. Values were considered to be statistically different when $P < 0.05$.

Results

Histology. Histological sections were studied from proximal, middle, and distal rings of venous grafts from six dogs. In all rings where the endothelium was not deliberately removed, endothelial cells lined the luminal surface (Fig. 2). All rings exhibited some degree of eccentric *myo*-intimal thickening (Table I). This thickening tended to be more prominent in distal portions of the venous grafts (Fig. 1) (Table I). The presence of stellate myofibroblasts was associated with the *myo*-intimal thickening (Fig. 1). Removal of the endothelium did not disrupt the intimal thickening (not shown).

Hemodynamic parameters. Mean arterial pressure measured was 129±4 mmHg ($n = 13$). The blood flows through the unoperated femoral arteries and the venous grafts were not significantly different (91.3±13.3 ml/min and 96.7±13.1 ml/min, respectively; $n = 12$).

Responsiveness of blood vessels. There were no differences in basal tension (tension at L_0) or maximal tension developed to KCl (60 mM) or norepinephrine (10^{-4} M) among rings of vein and venous graft with and without endothelium (Table II). Removal of the endothelium decreased significantly the ED_{50} to norepinephrine in unoperated veins. The responses to norepinephrine of rings of venous graft with and without endothelium were similar and comparable with those observed in rings of unoperated vein without endothelium (Table II).

Endothelium-dependent responses to acetylcholine. In rings of venous graft with or without endothelium, acetylcholine caused only increases in tension. The magnitude of the contractions of proximal, middle, and distal segments of venous graft were similar (Fig. 3). In contrast, concentration-dependent (10^{-8} to 3×10^{-7} M) decreases in tension to acetylcholine

Table II. Resting Tension and Contractile Responses in Unoperated Femoral Veins and Veins Grafted into the Femoral Artery

Blood vessel	Basal tension	60 mM KCl	Norepinephrine ED_{50}	Norepinephrine Tension to 10^{-4} M
	g	*g*	*−log M*	*g*
With endothelium				
Unoperated vein	1.2±0.2	2.6±0.4	6.2±0.1	5.0±0.3
Venous graft				
Proximal	2.5±0.5	1.9±0.3	6.6±0.1‡	3.0±0.5
Middle	2.1±0.2	2.1±0.2	6.7±0.1‡	3.5±0.5
Distal	1.5±0.3	2.0±0.3	6.7±0.1‡	4.3±0.9
Without endothelium				
Unoperated vein	1.6±0.5	1.8±0.4	6.6±0.1*	4.2±0.7
Venous graft				
Middle	2.2±0.3	1.9±0.3	6.6±0.1‡	3.1±0.5

Values presented as means±SEM; $n = 7$ in all groups.
* Denotes difference between unoperated veins with and without endothelium by Student's *t* test for paired observation; $P < 0.05$.
‡ Denotes difference from unoperated vein with endothelium by Scheffe's test for multiple comparisons; $P < 0.05$.

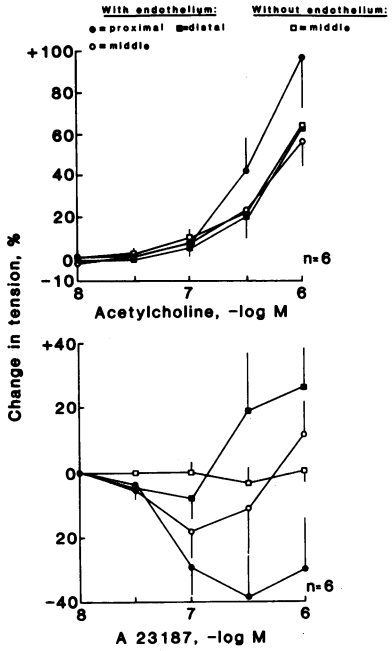

Figure 3. Responses in rings of venous grafts contracted with the ED_{30} of norepinephrine to acetylcholine (*top*) and the calcium ionophore, A23187 (*bottom*). Data are expressed as percent change from the response to norepinephrine (ED_{30}). Values are shown as means±SEM. No relaxations in rings with or without endothelium were observed to acetylcholine. The responses to A23187 were variable along the length of the graft. Endothelium-dependent relaxations were present in proximal segments of graft and tended to diminish in distal segments.

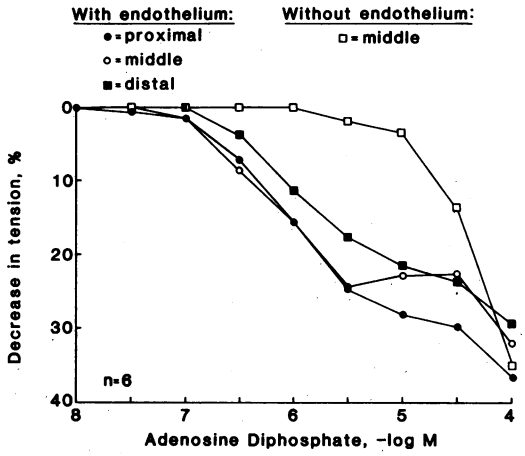

Figure 5. Relaxations in rings of venous graft contracted with the ED_{30} of norepinephrine to ADP. Data are shown as percent decrease from the response to norepinephrine (ED_{30}). Values are expressed as means; standard errors were omitted for the sake of clarity. There was significant variability among animals (two-way analysis of variance). At 3×10^{-6} M the relaxations in proximal and middle segments of graft with endothelium were significantly greater than in rings where the endothelium was removed (Scheffe's test for multiple comparisons; $P < 0.05$).

were observed in rings of unoperated veins with endothelium. At higher concentrations of the drug contractions were observed. In rings of unoperated vein without endothelium, only concentration-dependent (10^{-7} to 10^{-6} M) increases in tension were observed (Fig. 4).

ADP. In proximal, middle, and distal rings of venous grafts with endothelium, decreases in tension to ADP (10^{-7} to 10^{-4} M) were observed. At concentrations of ADP > 10^{-5} M, a relaxation of rings without endothelium was observed (Fig. 5).

Rings of unoperated veins relaxed in an endothelium-dependent manner to ADP with a maximal relaxation (−39.4±7.3% of the contraction to ED_{30} norepinephrine; $n = 6$) occurring at 3×10^{-6} M of the nucleotide. The relaxations at this concentration of ADP were not significantly different from those observed in proximal segments of venous grafts.

UK-14,304. In rings of venous grafts and unoperated veins contracted with prostaglandin $F_{2\alpha}$ (2×10^{-6} M), the selective α_2-adrenergic agonist UK-14,304 initiated concentration-dependent (10^{-9} to 10^{-6} M) further increases in tension (Fig. 6). The responses of the unoperated vein and venous graft segments with endothelium were not different from comparable rings without endothelium. The contractions of the venous

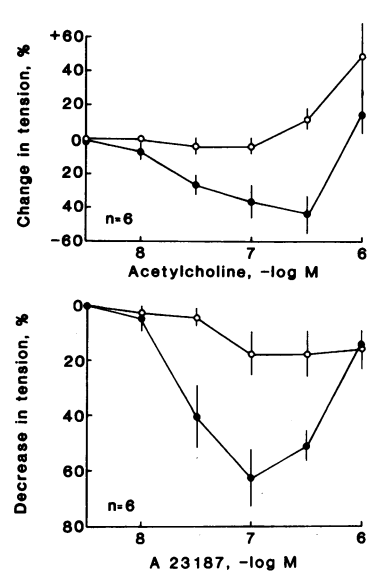

Figure 4. Responses in rings of canine femoral vein with (●) and without (○) endothelium contracted with ED_{30} of norepinephrine to acetylcholine (*top*) and the calcium ionophore A23187 (*bottom*). Data are shown as percent change from response to norepinephrine (ED_{30}). Values are expressed as means±SEM. Significant differences ($P < 0.05$) between rings with and without endothelium by Student's t test for paired observations were observed for both acetylcholine and calcium inophore at 3×10^{-8} to 3×10^{-7} M of the drugs.

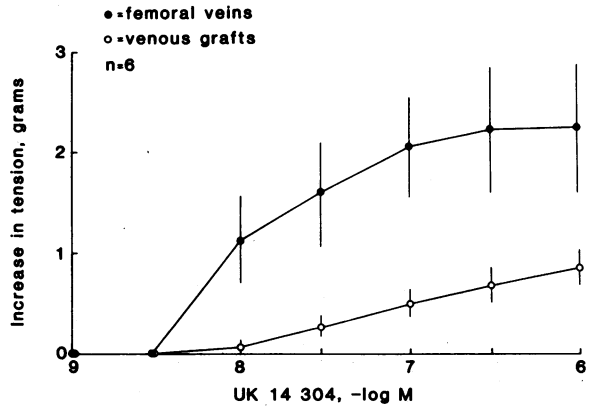

Figure 6. Contractions in rings without endothelium of canine femoral venous grafts and unoperated femoral veins to the α_2-adrenergic agonist, UK-14,304. Data are shown as increases in tension from the response to prostaglandin $F_{2\alpha}$: 1.8±0.2 g, femoral vein; 2.6±0.5 g, middle graft. Values expressed as means±SEM. The responses of femoral vein and graft segments with endothelium were not different from comparable rings without endothelium (data not shown). The contractions of segments of grafts with and without endothelium to UK-14304 were significantly less than those of the unoperated femoral vein at all concentrations of the agonist tested (Scheffe's test for multiple comparisons; $P < 0.05$).

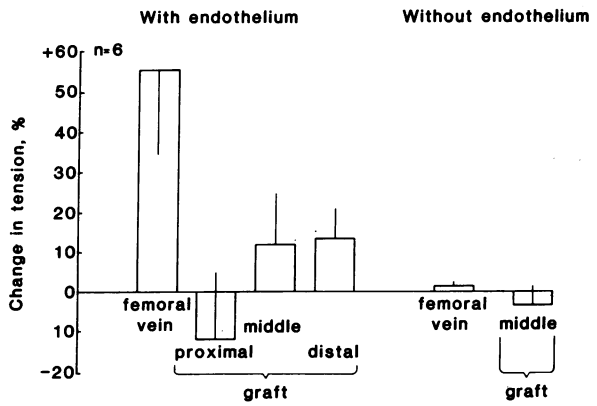

Figure 7. Responses of proximal segments of venous graft and unoperated femoral veins contracted with the ED_{30} of norepinephrine to arachidonic acid (10^{-6} M). In four of six experiments, relaxations in proximal segments of graft were observed. Data are shown as percent change from the response to norepinephrine (ED_{30}); values are expressed as means±SEM. The responses of proximal segments of graft and vein and graft segments without endothelium were significantly different from the responses of femoral vein with endothelium by Scheffe's test for multiple comparisons ($P < 0.05$).

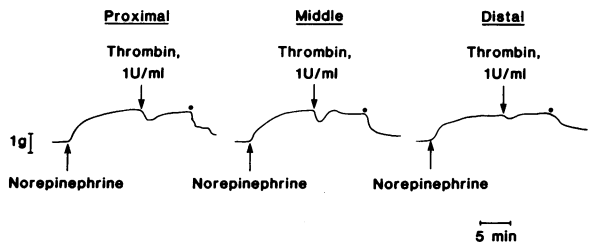

Figure 8. Endothelium-dependent responses to thrombin in rings of venous graft shown in Fig. 1. A significant loss of relaxation to thrombin occurred in distal segments of the graft where *myo*-intimal thickening was prominent. Drugs were removed from the organ chambers at (●).

grafts (with and without endothelium) to UK-14,304 were significantly less than those of the unoperated vein at all concentrations of the agonist tested (Fig. 6). The responses of proximal, middle, and distal segments of the graft were not significantly different (data not shown).

Arachidonic acid. The responses of the venous grafts to arachidonic acid (10^{-8} to 10^{-5} M) varied depending upon the position in the graft from where the ring was taken. Proximal rings of venous grafts with endothelium exhibited decreases in tension to 10^{-6} M arachidonic acid, whereas middle and distal rings of venous graft with endothelium exhibited only further increases in tension at this concentration of the fatty acid. No significant changes in tension were observed in segments of graft without endothelium (Fig. 7). However, at 10^{-5} M arachidonic acid all rings of grafts (with and without endothelium) exhibited only further increases in tension; these ranged from 80.1±16.2% to 102.9±24.1% (n = 6) of the initial contraction to norepinephrine (ED_{30}).

In rings of unoperated vein, arachidonic acid–initiated concentration-dependent increases in tension only when the endothelium was present. At 10^{-6} M arachidonic acid, these contractions were significantly greater than the responses of proximal segments of venous grafts (Fig. 7).

Thrombin. The responses of the venous grafts to thrombin (1 U/ml) also varied depending upon the anatomical origin of the ring. Four of seven proximal rings relaxed in response to thrombin. These relaxations averaged −47.7±8.8%, of the contraction to the ED_{30} norepinephrine. Contractions were observed in three rings. In rings from distal portions of the graft, relaxations when present were significantly less than in proximal segments (four of seven rings; −18.2±3.8% of the contraction to ED_{30} norepinephrine; Fig. 8). None of the rings of venous grafts without endothelium relaxed to thrombin; rather slight increases in tension were observed (25.2±13.1%, n = 7, of the contraction to the ED_{30} of norepinephrine). All rings of unoperated femoral veins with endothelium (seven of seven) relaxed to 1 U/ml of thrombin. These relaxations were transient and averaged −58.2±8.9% of the contraction to the ED_{30} of norepinephrine; in rings of unoperated vein without endothelium, no significant relaxations were observed (−6.6%±3.2, n = 7).

Calcium ionophore. The responses of venous graft to calcium ionophore (10^{-8} to 10^{-6} M) depended upon the location of the rings in the graft. In rings from the proximal portion of the grafts, endothelium-dependent decreases in tension were observed; these tended to diminish in the distal segments (Fig. 3). At 3×10^{-7} M the responses of proximal segments were significantly different from those of distal segments.

The ionophore caused endothelium-dependent decreases in tension in unoperated femoral veins at concentrations between 10^{-8} and 10^{-7} M; at higher concentrations contractions were observed (Fig. 4).

Indomethacin. Indomethacin did not affect the endothelium-dependent responses to acetylcholine, ADP, or calcium ionophore in either proximal rings of venous graft or unoperated veins (Table III).

Discussion

The morphological changes found in the femoral vein grafted into the arterial circulation in the present study are similar to those reported by others (5–9). Several factors may contribute to the nonuniform distribution of structural changes observed in the venous grafts. Surgical mobilization and anastomoses of vascular segments can result in denervation of those segments (10) and can lead to structural alteration of the smooth muscle (11). In addition disruption of the vasa vasorum, which can result in intimal thickening, probably occurred (5, 12–14). Further, although endothelialization of venous grafts is usually complete by 4–6 wk after surgery (7, 8), a combination of shear stress and turbulence are known to interfere endothelial cell adherence (15–17) and these factors may promote platelet-mediated hyperplasia and de-differentiation of the smooth muscle (9).

The presence of inhibitory endothelium-mediated responses seemed to follow the histological characteristics of the grafts. In proximal segments inhibitory responses to ADP, arachidonic acid, calcium ionophore, and thrombin prevailed.

The reasons for this are unclear. Although endothelial cells have been shown to regenerate in the direction of flow (18), there is no evidence that the endothelial cells in the proximal section of graft are of arterial origin.

A major finding of this study is that grafting of veins into the arterial circulation results in the selective loss of inhibitory

Table III. Effect of Indomethacin on Endothelium-dependent Responses in Venous Grafts and Unoperated Veins

Agent	Graft		Vein	
	Indomethacin (10^{-5} M) present	Absent	Present	Absent
Acetylcholine (3×10^{-7} M)	6.4±3.8*	10.9±6.2	−51.2±9.0	−63.4±10.4
ADP (3×10^{-6} M)	−24.8±6.9	−35.6±6.7	−32.8±7.0	−39.4±7.3
Calcium ionophore (10^{-7} M)	−49.1±10.9	−43.2±8.0	−70.4±8.4	−73.3±6.4

Experiments were conducted in parallel on paired rings of graft and vein in the absence and presence of indomethacin. * Values are shown as means±SEM of the percent change in tension from the contraction to the ED_{30} of norepinephrine; $n = 6$ in all groups.

endothelium-dependent responses to acetylcholine. The endothelial cells of venous grafts are capable of producing endothelium-derived relaxing factor(s) and the smooth muscle cells are capable of responding to such factor(s) because endothelium-derived inhibitory responses can be evoked by the calcium ionophore, A23187, a substance that releases a similar endothelium-derived factor as acetylcholine by a process not associated with receptor activation (1, 19). That not all receptor-mediated responses are lost in the endothelium of venous grafts is evidenced by the demonstration that ADP and thrombin evoke endothelium-dependent relaxations (2). The mechanism for the selective loss of the acetycholine-mediated relaxation in the venous grafts is unclear at this time. Chronic denervation of arterial vessels results in a blunting of the endothelium-dependent relaxation to muscarinic activation (20). Reduced relaxation to muscarinic activation also is observed in aortas of spontaneously hypertensive rats (21) and in aortic tissue in some models of experimental atherosclerosis (22–25). Arterial endothelial cells grown in culture do not release relaxing factors to muscarinic activation, a response that returns when the cells are co-cultured with smooth muscle cells (26, 27).

Two products of arachidonic acid metabolism, prostacyclin and thromboxane, are altered in saphenous or jugular veins grafted into the carotid arteries of the dog (8, 28). Although the chemical nature of endothelium-derived inhibitory substance(s) in venous grafts was not identified in this study, it is unlikely that the substance(s) released in response to ADP or calcium ionophore is a product of the metabolism of arachidonic acid by cyclo-oxygenase. An inhibitor of cyclo-oxygenase, indomethacin did not diminish endothelium-dependent relaxations in rings of unoperated vein or venous graft to these agents.

The inhibitory responses to arachidonic acid in proximal segments of graft resembles those of unoperated arteries (29), whereas contractile responses of the distal segments are characteristic of unoperated veins (29, 30). These results suggest that the endothelial cells of the proximal segments may be of arterial origin and thus retain the characteristics of the mature blood vessel. Alternatively, the end products of the metabolism of arachidonic acid by the regenerated venous endothelial cells may be modified or the sensitivity of the venous smooth muscle cells to a given end product may be altered. The responses to exogenous arachidonic acid are mediated through the cyclo-oxygenase pathway in both arteries and veins (30, 31).

Even though the venous grafts are exposed to arterial levels of blood flow, pressure, and oxygen tension, the changes in endothelium-dependent responses of the graft are not the same as those observed in veins proximal to an arteriovenous fistula (3). One difference, already discussed, is that this endothelium-dependent relaxation to acetylcholine is lost in venous grafts, a response which is enhanced in veins proximal to a fistula. A second is that the difference in sensitivity to norepinephrine between venous rings with and without endothelium (preserved in veins of a fistula) is absent in rings of venous grafts. Increased sensitivity to norepinephrine was observed also in saphenous vein segments grafted into the femoral artery (32). Whether this increased sensitivity to adrenergic stimulation of the venous grafts with endothelium is due to a loss of tonic release of endothelium-derived relaxing factor, loss of endothelial adrenergic receptors (4, 33), or a combination of these is unclear. An increased contractile response due to smooth muscle proliferation is unlikely as the contractile response to potassium chloride in the femoral (present study, Table I) and saphenous (32) venous grafts was unaltered.

Endothelial α_2-adrenergic relaxations are difficult to demonstrate in veins (4). These responses seem to be more prevalent in tissues exposed to high flow and oxygen tensions. In veins proximal to a fistula where blood flow and oxygen tension are elevated, endothelium-dependent relaxations to α_2-adrenergic stimulation can be observed (3). The loss of endothelial adrenergically mediated responses of venous grafts is suggested by the similarity of concentration-response curves to norepinephrine in tissues with and without endothelium and by the absence of the stimulated release of inhibitory factors to UK-14,304 in rings contracted with prostaglandin $F_{2\alpha}$. Therefore, the loss of responsiveness to α_2-adrenoceptor stimulation of the endothelial cells in venous grafts suggests that this receptor-mediated response is lost as is that response to acetylcholine.

The endothelial α_2-adrenergic relaxations were absent despite a diminished α_2-adrenergic stimulation of the venous smooth muscle. α_2-adrenoceptors on the smooth muscle seem to predominate in those portions of the circulation where pH and oxygen tension are low (34). The venous grafts were exposed to arterial levels of pH and oxygen tension. Therefore, the reduced α_2-adrenoceptor mediated contractions of the smooth muscle suggest that in addition to endothelial responses, the smooth muscle contractility can also be modulated by local environmental factors.

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