Influence of tangential stress on mechanical responses to vasoactive agents in human saphenous vein with and without perivascular adipose tissue

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AIM: Improvement in short-term patency of vein grafts harvested with the surrounding tissue and no distention has been noted. The influence of transient tangential stress on mechanical function to vasoactive agents in isolated human saphenous veins stripped or with attached perivascular adipose tissue was assessed.

METHODS: Concentration-response curves to noradrenaline, 5-hydroxytryptamine, methylcholine, sodium nitroprusside and nicardipine were constructed for veins exposed to no, low (approximately 120 mmHg) or high (approximately 240 mmHg) tangential stress.

RESULTS: Tangential stress did not affect contractile effects of noradrenaline or relaxant effects of methylcholine and sodium nitroprusside. Regression analysis of the concentration-response curve to 5-hydroxytryptamine revealed a significant ($P=0.042$) increase in sensitivity in saphenous veins without perivascular adipose tissue exposed to no tangential stress, compared with veins with attached adipose tissue. Exposure to high stress significantly ($P=0.024$) increased the potency of 5-hydroxytryptamine in blood vessels without perivascular adipose tissue, as opposed to veins with adipose tissue. Relaxant responses to nicardipine in veins with perivascular adipose tissue were significantly ($P=0.001$) affected by exposure to low tangential stress compared with no or high tangential stress. A parallel comparison revealed that intact veins compared with those without perivascular adipose tissue exposed to low stress were significantly ($P=0.020$) more resistant to the relaxant effects of nicardipine.

CONCLUSION: The findings of the present report support the view that tangential stress has an impact on the actions of vasoactive agents, but this influence is variable and factor(s) released from perivascular adipose tissue may have a bearing on the observed effect.

Key Words: *Adipose tissue; Blood vessel; Contraction; Relaxation; Tangential stress; Vasoactive agents*

Saphenous vein grafts with very low blood flow are believed to occlude quickly as a result of thrombus formation triggered by endothelial damage (1). The overall occlusion rate of saphenous vein grafts within six months can be greater than 20% (2), and comparatively, the rate of attrition of saphenous vein grafts seems to greatly exceed that of internal mammary artery grafts over 10 years postoperation (3). Approximately 39% of saphenous vein grafts occlude within 10 years, while

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BUT : Une amélioration de la perméabilité, à court terme, de greffons veineux prélevés avec du tissu circonférentiel, non distendus, a déjà été observée. Nous avons donc évalué l'action de contraintes transitoires de cisaillement sur les réactions mécaniques de veines saphènes humaines, isolées, dépouillées ou non de tissu adipeux périvasculaire, à des agents vasomoteurs.

MÉTHODE : Il y a eu mesure des courbes concentration-réaction à la noradrénaline, à la 5-hydroxytryptamine, à la méthylcholine, au nitroprussiate de sodium et à la nicardipine dans des veines exposées à des contraintes de cisaillement nulles, faibles (environ 120 mm Hg) ou élevées (environ 240 mm Hg).

RÉSULTATS : Les contraintes de cisaillement n'ont pas modifié l'effet de contraction de la noradrénaline ni l'effet de relaxation de la méthylcholine ou du nitroprussiate de sodium. Une analyse de régression de la courbe concentration-réaction à la 5-hydroxytryptamine a révélé une augmentation significative ($p = 0,042$) de la sensibilité des veines saphènes dépouillées de tissu adipeux périvasculaire, exposées à une contrainte de cisaillement nulle, par rapport aux veines saphènes enveloppées de tissu adipeux. L'exposition à une contrainte élevée a eu pour effet d'augmenter de façon significative ($p = 0,024$) la puissance d'action de la 5-hydroxytryptamine dans des vaisseaux sanguins dépouillés de tissu adipeux périvasculaire par rapport à ceux qui ne l'étaient pas. La réaction de relaxation des veines enveloppées de tissu adipeux périvasculaire à la nicardipine a été significativement ($p = 0,001$) modifiée par l'exposition à une contrainte de cisaillement faible par rapport à des contraintes de cisaillement nulles ou élevées. Une comparaison parallèle a révélé que les veines enveloppées de tissu adipeux périvasculaire, exposées à une contrainte de cisaillement faible étaient significativement ($p = 0,020$) plus résistantes à l'effet de relaxation de la nicardipine que les veines dépouillées.

CONCLUSION : Les résultats de l'étude confirment l'opinion selon laquelle les contraintes de cisaillement modifient l'action d'agents vasomoteurs, mais leur effet est variable, et la libération d'un certain facteur du tissu adipeux périvasculaire pourrait influencer sur l'effet observé.

only 10% to 12% of internal mammary arteries occlude over this course (3).

It has been postulated that an impairment in the synthesis and release of endothelium-derived relaxing factors could play a critical role in the higher incidence of atherosclerosis and occlusion in saphenous vein grafts (4). There is some evidence suggesting that perivascular tissue stripping and venous distention contribute to the disruption of the endothelial cell lining

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of the vessel (5,6) and lead to a reduction in nitric oxide synthase levels in the conventionally harvested veins (6,7). Additionally, it has been suggested that preservation of medial vascular smooth muscle cells of the saphenous vein may also contribute to improved early patency rate (8). Evidently, despite the variety of reasons that have been put forward to explain the high failure rate of saphenous vein grafts in situ, there is no real consensus on this issue.

However, of special interest is the observation of improvement in the short-term patency of vein grafts harvested with the surrounding tissue and no distention (9). This observation seems to imply that vein grafts implanted with surrounding tissue and no distention may possess physiological properties that, at least in the short term, preclude occlusion of the vessels (9). Therefore, in the present investigation, we attempted to systematically assess the influence of tangential stress on mechanical function induced by a number of vasoactive agents in isolated human saphenous veins. We also tried to assess whether functional changes to actions of endogenous or exogenous mediators are altered by distention and/or presence and absence of perivascular adipose tissue. To this end, the effects of noradrenaline, 5-hydroxytryptamine, methylcholine (endothelium-dependent vasodilator), sodium nitroprusside (endothelium-independent vasodilator) and nicardipine (L-type calcium channel antagonist) were examined in isolated human saphenous veins, either with or without perivascular adipose tissue, and were then exposed transiently to two levels of tangential stress.

METHODS

Experiments conducted for the present investigation conform with the Canadian Institutes of Health Research policies and guidelines in ethics. The protocol for these experiments was approved by the Human Investigation Committee, Memorial University of Newfoundland in St John's, Newfoundland and Labrador.

Tissue isolation

Experiments on saphenous veins for all the studies described were performed on the same day they were taken from patients. Segments of saphenous veins were obtained from 48 patients (37 men and 11 women, age range 35 to 77 years) undergoing coronary artery bypass surgery. The tissues were placed in ice-cold physiological salt solution immediately after leaving the operating room. The composition of the physiological salt solution included 132 mM sodium chloride, 4.6 mM potassium chloride, 1.2 mM magnesium chloride, 2.5 mM calcium chloride, 1.2 mM potassium dihydrogen phosphate, 12.5 mM sodium bicarbonate, 0.1 mM ethylenediaminetetraacetic acid and 11.0 mM glucose. The veins with or without perivascular adipose tissue were exposed to no, low or high tangential stress. In an intact segment, one end was tied using a 4-0 silk suture, while an 18 gauge catheter was inserted into the other end and tied, and then attached to a mercury manometer. In vessels that were distended, pressure was raised and maintained for 1 min. The mean $\pm$ SD low stress for tissues with and without perivascular adipose tissue was 123.80 $\pm$ 2.96 mmHg and 122.00 $\pm$ 1.20 mmHg, respectively, while the mean $\pm$ SD high stress for tissues with and without perivascular adipose tissue was 245.10 $\pm$ 6.10 mmHg and 245.00 $\pm$ 4.50 mmHg, respectively. The pressure was released and the blood vessel was cut into rings. Saphenous vein rings (2 mm in length) were then mounted for mechanical studies in organ

Mechanical studies

Rings were mounted in 15 mL organ baths at 36 $\pm$ 1°C, continuously gassed with a mixture of 95% O₂ and 5% CO₂, and were placed under a force of 19.6 mN (10). The tissues were equilibrated for 90 min (washed twice at 30 min and 60 min with physiological salt solution). Isometric tension was measured with force displacement transducers (model FT03, Grass Instruments Co, USA) connected to a polygraph (model 7PCB, Grass Instruments Co, USA). All tissues were contracted with a 80 mM potassium solution after the initial 90 min of equilibration, then washed with regular physiological salt solution and allowed to equilibrate for a further 60 min. Subsequently, concentration-response curves to noradrenaline (1 nM to 10 μ M), 5-hydroxytryptamine (1 nM to 10 μ M), methylcholine (1 nM to 30 μ M), sodium nitroprusside (1 nM to 30 μ M) and nicardipine (1 nM to 30 μ M) were constructed. In experiments in which relaxation was studied, tissues were contracted with noradrenaline before the addition of the agonists (ie, methylcholine, sodium nitroprusside and nicardipine).

Chemicals

Stock solutions of all drugs, except nicardipine, were made in twice-distilled water. A stock solution of 0.1 M nicardipine was made in 0.1 M hydrochloric acid and subsequently diluted in twice-distilled water. All drugs were purchased from Sigma RBI Chemical Company (Canada).

Data and statistical analysis

The experimental design and analyses were unbiased, and no group assignments were done according to patient sex, disease state or drug history. Results from contraction and relaxation studies were calculated as the percentage of maximum response induced by the agonist, and pEC₅₀ (the negative logarithm₁₀ of the effective concentration [EC₅₀]) values were obtained for individual curves by means of a computer program (11). In addition, polynomial regression analysis for obtaining the slope was carried out for individual concentration-response curves (at EC₂₀ to EC₈₀) for agonists except for methylcholine. In the latter case, it was not possible to effectively apply regression analysis to assess the slope of the plots due to the shallow nature of the concentration-response curve.

ANOVA on ranks was used for comparisons between means, and Student-Newman-Keuls test was used for pairwise comparisons to identify differences between individual means. Student's unpaired *t* test was also used when comparisons were made between two means. For all cases, a probability of error of less than 0.05 was selected as the criterion for statistical significance. Unless noted otherwise, data are presented as the mean $\pm$ SEM.

RESULTS

Absolute maximal tension elicited by noradrenaline in human saphenous vein ring preparations was found not to be significantly different in veins with (P=0.136) or without (P=0.514) perivascular adipose tissue after exposure to no, low or high tangential stress. However, it seems that intact veins exposed to high tangential stress, as opposed to no or low tangential stress, had a tendency to develop less maximal tension to noradrenaline (Table 1). The maximal developed tension to 5-hydroxytryptamine was also found not to be significantly different in vessels with (P=0.473) or without (P=0.526) perivascular adipose tissue exposed to no, low or high tangential stress (Table 1).

TABLE 1
Absolute maximal tension in human saphenous vein ring preparations to noradrenaline and 5-hydroxytryptamine exposed to no, low or high tangential stress*

	No stress	Low stress	High stress
With perivascular adipose tissue			
Noradrenaline	44.55±13.22	51.17±10.86	19.29±4.04
5-hydroxytryptamine	45.21±14.10	36.79±7.68	26.16±5.64
Without perivascular adipose tissue			
Noradrenaline	28.2±4.15	37.36±9.54	26.32±4.11
5-hydroxytryptamine	31.10±5.68	21.91±7.70	21.5±4.15

Each value is a mean of six experiments ± SEM. *Average (mean ± SD) low stress in veins with and without perivascular adipose tissue was 123.80±2.96 mmHg and 122.00±1.20 mmHg, respectively; average high stress for veins with and without perivascular adipose tissue was 245.10±6.10 mmHg and 245.00±4.50 mmHg, respectively

TABLE 2
The negative logarithm₁₀ of the effective concentration values for concentration-response curves for various vasoactive agents in human saphenous vein exposed to no, low or high tangential stress*

	No stress	Low stress	High stress
With perivascular adipose tissue			
Noradrenaline	7.18±0.06	7.11±0.10	7.07±0.08
5-hydroxytryptamine	6.69±0.05	6.94±0.06	7.00±0.11†
Methylcholine	6.72±0.31	7.30±0.29	7.12±0.38
Sodium nitroprusside	6.89±0.07	6.82±0.15	6.76±0.23
Nicardipine	5.89±0.27	6.43±0.65	5.31±0.40
Without perivascular adipose tissue			
Noradrenaline	7.31±0.09	7.31±0.04	7.20±0.09
5-hydroxytryptamine	7.00±0.18	7.17±0.10	7.40±0.08
Methylcholine	6.95±0.15	7.19±0.22	7.09±0.21
Sodium nitroprusside	6.75±0.29	7.24±0.20	7.26±0.12
Nicardipine	6.14±0.63	5.27±0.54	5.90±0.58

Each value is a mean of six experiments ± SEM. *Average (mean ± SD) low stress in veins with and without perivascular adipose tissue was 123.80±2.96 mmHg and 122.00±1.20 mmHg, respectively; average high stress for veins with and without perivascular adipose tissue was 245.10±6.10 mmHg and 245.00±4.50 mmHg, respectively; †Significantly different from respective value without perivascular adipose tissue

Effects of noradrenaline and 5-hydroxytryptamine

Contractions elicited by noradrenaline were not affected by the presence of perivascular adipose tissue compared with no perivascular adipose (Table 2, Figure 1A-C). Furthermore, tangential stress did not have a significant impact on responses to noradrenaline in saphenous veins with (P=0.201) or without (P=0.640) perivascular adipose tissue (Table 3).

Contractions elicited by 5-hydroxytryptamine did not seem to be affected by the presence or absence of perivascular adipose tissue in saphenous rings exposed to low stress (Tables 2 and 3, Figure 2B). However, regression analysis of the concentration-response curve to 5-hydroxytryptamine revealed a steeper slope and a significant (P=0.042) increase in the sensitivity to this monoamine in saphenous veins exposed to no tangential stress without perivascular adipose

Figure 1 Concentration-response curves to noradrenaline in human saphenous vein ring preparations with (closed circles) or without (open circles) perivascular adipose tissue exposed to no (A), low (B) or high (C) tangential stress. Each value is a mean of six experiments ± SEM. Average (mean ± SD) low stress for veins with and without perivascular adipose tissue were 123.80±2.96 mmHg and 122.00±1.20 mmHg, respectively, while average high stress for veins with and without perivascular adipose tissue were 245.10±6.10 mmHg and 245.00±4.50 mmHg, respectively

tissue, compared with the intact tissue (Table 3, Figure 2A). High stress also significantly (P=0.024) increased the potency of 5-hydroxytryptamine in veins without perivascular adipose tissue, as opposed to vessels with adipose tissue (Table 2, Figure 2C).

TABLE 3

Slope values and regression coefficient of concentration-response curves (EC_{20} to EC_{80}) for various vasoactive agents in human saphenous vein exposed to no, low or high tangential stress*

Agonists	No stress	Low stress	High stress
	With perivascular adipose tissue		
Noradrenaline	0.038±0.004 (0.997)	0.039±0.009 (0.998)	0.088±0.030 (0.998)
5-hydroxytryptamine	0.043±0.003 [†] (0.986)	0.045±0.005 (0.991)	0.043±0.007 (0.982)
Sodium nitroprusside	0.055±0.003 (0.990)	0.060±0.007 (0.987)	0.115±0.031 (0.986)
Nicardipine	1.10±0.284 [‡] (0.983)	13.00±2.50 [†] (0.995)	2.99±1.40 [‡] (0.987)
Agonists	Without perivascular adipose tissue		
	No stress	Low stress	High stress
Noradrenaline	0.039±0.010 (0.998)	0.039±0.008 (0.999)	0.057±0.020 (0.995)
5-hydroxytryptamine	0.074±0.012 (0.986)	0.045±0.007 (0.997)	0.067±0.012 (0.991)
Sodium nitroprusside	0.202±0.069 (0.980)	0.082±0.009 (0.971)	0.065±0.016 (0.990)
Nicardipine	0.858±0.219 (0.986)	3.26±1.94 (0.977)	3.21±1.50 (0.999)

Each value is a mean of six experiments ± SEM. *Average (mean ± SD) low stress in veins with and without perivascular adipose tissue were 123.80±2.96 mmHg and 122.00±1.20 mmHg, respectively; average high stress for veins with and without perivascular adipose tissue were 245.10±6.10 mmHg and 245.00±4.50 mmHg, respectively; [†]Significantly different from respective value without adipose tissue; [‡]Significantly different from low stress

Effects of methylcholine and sodium nitroprusside

Concentration-response curves to methylcholine were fairly shallow in saphenous vein rings with or without perivascular adipose tissue (Figure 3A-C). Tangential stress did not have a significant impact on endothelium-dependent relaxation in intact vessels or those from which perivascular adipose tissue had been removed (Table 2). However, there was a tendency for the vessels without perivascular adipose tissue to relax more than those with the tissue following exposure to high stress (Figure 3C). However, the latter was found not to be significant.

Endothelium-independent relaxation to sodium nitroprusside was not affected by the presence or absence of perivascular adipose tissue (Tables 2 and 3, Figure 4A-C). Although exposure of the blood vessels to tangential stress at low and high stress levels had a tendency to increase the relaxant responses to sodium nitroprusside in veins without perivascular adipose tissue, as opposed to those with adipose tissue, the events were found to be insignificant ($P=0.166$ and $P=0.119$, respectively).

Effects of nicardipine

Relaxant responses to nicardipine in blood vessels with perivascular adipose tissue were significantly ($P=0.001$) affected by exposure to low tangential stress compared with no stress or high stress (Table 3). Regression analysis of the concentration-response curves revealed that exposure to low tangential stress in intact blood vessels resulted in a shallower slope and a significant reduction in relaxation to nicardipine compared with the effects of the calcium channel antagonist in tissue exposed to no or high tangential stress (Table 3). In addition, parallel comparisons revealed that intact veins exposed to low tangential stress, as opposed to those without perivascular adipose tissue, were significantly ($P=0.020$) more resistant to the relaxant effects of nicardipine (Table 3, Figure 5B). In contrast, a similar parallel comparison revealed that no tangential stress ($P=0.547$) or high tangential stress ($P=0.937$) had limited effects on the relaxant responses to nicardipine in intact blood vessels as opposed to those without perivascular adipose tissue (Table 3, Figure 5A,C).

DISCUSSION

In the present investigation, the level assigned to the low tangential stress was a distention that the vein graft could experience in situ, while the high level was one that may not be ordinarily experienced in situ. Wendling et al (12) had reported that tension applied to human saphenous vein rings to produce distention of 200 mmHg to 300 mmHg using La Place's law resulted in a reduced maximal response to the selective α_1 -adrenoceptor agonist, phenylephrine, without altering the concentration-response curve of the latter. Furthermore, the concentration-response curves to high potassium buffer or the calcium channel antagonist, isradipine, were also not affected by similar distention in the saphenous vein ring preparations (12).

Overall, it is apparent that the levels of transient stress applied in the present investigation do not have a significant impact on mechanical force generated by noradrenaline. This is in contrast to the impact that distention had on 5-hydroxytryptamine-elicited tensions. In blood vessels without perivascular adipose versus intact tissue, high tangential stress increased the sensitivity to the development of tension by 5-hydroxytryptamine. It is possible that the presence of perivascular adipose tissue restricted the expansion of the venous smooth muscle cells, making the vein less responsive to the actions of this monoamine. This may not be of great consequence in a graft in situ if the subject's blood pressure does not become elevated to high levels. However, in stripped tissue, even a marginal increase in sensitivity to the vasoconstrictive actions of 5-hydroxytryptamine (triggered by transient elevation in pressure in the grafted conduit), coupled with its physiological actions on platelets (ie, aggregation), may provide a critical trigger in the reduction of flow in the vein grafts and could result in occlusions over time. Moreover, it is evident that blood vessels without perivascular adipose exposed to no tangential stress, as opposed to those with intact tissue, were significantly more sensitive to the actions of 5-hydroxytryptamine. This further enforces the idea that the lack of perivascular adipose tissue seems to accentuate the vasoconstrictive effects of this monoamine.

Figure 2) Concentration-response curves to 5-hydroxytryptamine in human saphenous vein ring preparations with (closed circles) or without (open circles) perivascular adipose tissue exposed to no (A), low (B) or high (C) tangential stress. Each value is a mean of six experiments $\pm$ SEM. Average (mean $\pm$ SD) low stress for veins with and without perivascular adipose tissue were 123.80 ± 2.96 mmHg, and 122.00 ± 1.20 mmHg, respectively, while average high stress for veins with and without perivascular adipose tissue were 245.10 ± 6.10 mmHg, and 245.00 ± 4.50 mmHg, respectively

The likelihood that perivascular adipose tissue is capable of influencing vascular tone remains a possibility in the present investigation. Lohn et al (13) reported that maximal responses to agonists such as angiotensin II, 5-hydroxytryptamine and phenylephrine were attenuated by 95%, 80% and 30%, respectively, in isolated rat aortic rings with perivascular adipose

Figure 3) Concentration-response curves to methylcholine in human saphenous vein ring preparations with (closed circles) or without (open circles) perivascular adipose tissue exposed to no (A), low (B) or high (C) tangential stress. Each value is a mean of six experiments $\pm$ SEM. Average (mean $\pm$ SD) low stress for veins with and without perivascular adipose tissue were 123.80 ± 2.96 mmHg, and 122.00 ± 1.20 mmHg, respectively, while average high stress for veins with and without perivascular adipose tissue were 245.10 ± 6.10 mmHg, and 245.00 ± 4.50 mmHg, respectively

tissue compared with vessels without the tissue. It has thus been suggested that factor(s) released from adipocytes, called adipocyte-derived relaxing factor (ADRF), is responsible for influencing the actions of vasoactive agents. Moreover, the influence of perivascular adipose tissue was found to be independent of nitric oxide production or the presence of

Figure 4) Concentration-response curves to sodium nitroprusside in human saphenous vein ring preparations with (closed circles) or without (open circles) perivascular adipose tissue exposed to no (A), low (B) or high (C) tangential stress. Each value is a mean of six experiments $\pm$ SEM. Average (mean $\pm$ SD) low stress for veins with and without perivascular adipose tissue was 123.80 ± 2.96 mmHg, and 122.00 ± 1.20 mmHg, respectively, while average high stress for veins with and without perivascular adipose tissue was 245.10 ± 6.10 mmHg, and 245.00 ± 4.50 mmHg, respectively

endothelium (13). In the present study, it is possible that the increased sensitivity to 5-hydroxytryptamine in intact tissues resulted from the release of ADRF. The fact that ADRF did not appear to influence responses to noradrenaline may indicate that different signalling processes were activated by noradrenaline

Figure 5) Concentration-response curves to nicardipine in human saphenous vein ring preparations with (closed circles) or without (open circles) perivascular adipose tissue exposed to no (A), low (B) or high (C) tangential stress. Each value is a mean of six experiments $\pm$ SEM. Average (mean $\pm$ SD) low stress for veins with and without perivascular adipose tissue was 123.80 ± 2.96 mmHg, and 122.00 ± 1.20 mmHg, respectively, while average high stress for veins with and without perivascular adipose tissue was 245.10 ± 6.10 mmHg, and 245.00 ± 4.50 mmHg, respectively

that were not affected by the release of ADRF. It is evident that the release of such factors from adipose tissue has a variable impact on contractions elicited by different agents. It seems that the least inhibitory influence was exerted on responses mediated via the activation of α_1 -adrenoceptors

by the application of phenylephrine (13), and may explain why ADRF did not significantly affect responses to noradrenaline in the present study. While the inhibitory actions of ADRF in rat aortic rings were suggested to be via the tyrosine kinase pathways and the opening of ATP-sensitive potassium channels (13), similar inhibitory effects in rat isolated mesenteric arteries were suggested to involve the activation of voltage-dependent potassium channels (14). In addition, it has also been suggested that the release of ADRF in rat isolated aortic rings was dependent on calcium and 3', 5' cyclic AMP (15).

The lack of significant change to the relaxant actions of methylcholine simply implies that receptor- and endothelium-dependent relaxation is not greatly influenced by the level of stress and/or presence of perivascular adipose tissue in this paradigm. It is recognized that endothelium-dependent relaxation in the human saphenous vein is quite modest (16,17). Shapira et al (18) reported that endothelium-dependent relaxation is weakest in the saphenous vein, compared with the internal mammary artery and the radial artery. In addition, nitroglycerine-stimulated increases in the levels of cyclic GMP is also significantly lower in the human saphenous vein than in the radial artery or the internal mammary artery (18). The fact that the basal levels of cyclic GMP are also lower in the saphenous vein than in the radial artery or the internal mammary artery (18) perhaps indicates that nitric oxide does not play as prominent a role in the control of vascular muscle in this vessel as with the arteries mentioned. Liu et al (19) have also reported that basal release of nitric oxide and endothelium-dependent hyperpolarizing factors are significantly lower in saphenous vein than internal mammary artery. It is possible that although the level of distention in the present investigation did not have a significant effect on receptor-mediated relaxation, it may have had an impact on the ability of nitric oxide synthase to function optimally. Certainly, it has been suggested that tissue stripping and venous distention both contribute to reduced nitric oxide synthase levels, while the 'no-touch' technique may aid in maintaining intact endothelium (5,6) and the preservation of the enzyme in the saphenous vein (6,7).

In the present investigation, there was a tendency for a modest but insignificant increase in sensitivity to the relaxant actions of sodium nitroprusside in saphenous veins without perivascular adipose tissue exposed to low or high tangential stress, as opposed to veins with perivascular adipose tissue. It was also interesting to note that vessels exposed to high tangential stress without perivascular adipose tissue, compared with intact tissue, appeared to be modestly but insignificantly more sensitive to the relaxant effects of methylcholine.

The relaxant response to nicardipine was influenced by the presence of perivascular adipose tissue, as well as distension. It seems that the low tangential stress significantly impeded the relaxant actions of nicardipine, and this became more evident in blood vessels with perivascular adipose tissue. It is difficult to readily explain why the impact of low tangential stress in intact veins reduced the relaxant actions of nicardipine. However, it is plausible that the combination of low tangential stress and perivascular adipose tissue reduced the participation of L-type calcium channels in the process of excitation-contraction coupling. Given that high tangential stress seemed to reverse this event and revert back the effectiveness of nicardipine in vessels with perivascular adipose tissue, it may be argued that in the latter vessels, the functional participation of L-type calcium channels are again making a significant

contribution to the maintenance of active tension induced by the agonist (ie, noradrenaline). Although the vasorelaxant actions of some calcium channel antagonists (eg, nifedipine, amlodipine), have been found, in part, to be dependent on the presence of functional endothelial cells and sensitive to the action of nitric oxide synthase inhibitors (20), the vasodilatory effect of nicardipine has been found not to be mediated via the generation of nitric oxide, at least via its activation, in healthy human subjects (21). Accordingly, based on our current observations with nicardipine, this event could be of clinical importance in situ because calcium channel antagonists (eg, nicardipine) have been used as vasodilators in patients who have undergone coronary artery bypass surgery (22).

SUMMARY

Our findings seem to indicate that both tangential stress and/or perivascular adipose tissue can have a profound effect on the mechanical actions of some vasoactive agents. While these effects were significant in the cases of 5-hydroxytryptamine and nicardipine, the activities of noradrenaline, methylcholine and sodium nitroprusside were not significantly affected by tangential stress and/or the presence of perivascular adipose tissue.

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