Inhibition of Adrenergic Vasoconstriction by Endothelial Cell Shear Stress

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Isolated perfused rabbit carotid arteries were used to determine the effects of endothelial cell shear stress on the response to adrenergic nerve stimulation. Arterial segments with and without endothelium were cannulated and perfused with physiological salt solution. Adrenergic nerves were activated by transmural electrical field stimulation. Neurogenic vasoconstriction was significantly greater in segments without endothelium when compared with that of segments with endothelium. In segments with endothelium only, vasoconstriction was depressed when shear stress was increased by increasing the viscosity of the perfusate with dextran. Perfusion with methylene blue (2 × 10⁻⁶ M), a guanylate cyclase inhibitor, increased vasoconstriction in segments with endothelium only. In the presence of methylene blue, vasoconstriction was no longer different between segments with and without endothelium, and perfusion with dextran had no effect. In a perfusion-cascade system, perfusion with dextran of donor segments with but not without endothelium caused further relaxation of a contracted basilar ring. These results suggest that shear stress on endothelial cells modulates adrenergic vasoconstriction by augmenting release of endothelial cell-derived vasodilators. (Circulation Research 1988;63:720–725)

Endothelial cells have been shown to exert an influence on vascular smooth muscle tone by releasing an endothelium-derived relaxing factor (EDRF) in response to various agents. EDRF is also released spontaneously and thus may inhibit adrenergic neurogenic vasoconstriction.

Endothelial cell-mediated vasodilation in response to marked increases in blood flow has been reported in canine coronary and femoral arteries in situ. A possible interpretation of these studies is that elevations in shear stress caused by increases in blood flow through an artery triggers release of EDRF from the endothelium. Experiments with perfusion-cascade preparations demonstrate that increases in perfusate flow induce the release of relaxing substances from the endothelium. Endothelial cells in culture respond to varying shear stress by changes in their function, and there is a correlation between prostacyclin release and shear stress. The aim of the present study was to determine the effects of increasing endothelial cell shear stress on the vasoconstriction caused by adrenergic nerve stimulation.

Materials and Methods

The carotid artery was dissected from male New Zealand white rabbits (2.5 kg) killed by exsanguination after anesthesia with sodium pentobarbital (30 mg/kg i.v.). The adhering perivascular tissue was carefully removed. Side branches were ligated with 4-0 surgical sutures. Segments of arteries (1.5 cm long) were mounted onto inflow and outflow cannulae in a vessel chamber with a volume of 25 ml. Segments were checked for leaks by applying a low pressure with a syringe and outflow cannulae was perfused with physiological salt solution (PSS) of the following millimolar concentrations: NaCl 118.3, MgSO₄ 0.6, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25.0, calcium ethylenediamine tetraacetic acid 0.026, and glucose 11.1.

Flow was measured with an electromagnetic flowmeter (model BL 610, Biotronex, Kensington, Maryland). The flowmeter was calibrated with volume collected over a period of time, and zero flow was rechecked frequently during the experiments. An adjustable stopcock was used to change outflow resistance. The inflow and outflow pressure was measured with a pressure transducer (Gould-Statham, Cleveland, Ohio). The inflow pressure was kept constant with an electronic servo-control system. PSS heated to 37 ± 0.5°C and oxygenated with 95% O₂-5% CO₂ was perfused through the...
artery from a separate reservoir with a peristaltic pump with a pulse dampener (Cole-Parmer, Chicago, Illinois). The vessel length was set by pressurizing the vessel with the outflow cannula closed so that the buckle of the vessel was just removed at a transmural pressure of 90 mm Hg. This length was found to be comparable to the length of the vessel in situ. The artery was then pressurized to 25 mm Hg for a 1-hour equilibration period.

Constrictor responses to electrical stimulation were determined at various transmural pressures (20–120 mm Hg). In this preparation, vasoconstriction was largest at a transmural pressure of 40 mm Hg. Thus, the studies were performed at this transmural pressure to optimize responses.

Removal of Endothelium

The endothelium was removed with 0.3% wt/vol of 3-[3-cholamidopropyl]-dimethylamino-1-propanesulfonate (CHAPS), perfused through the artery segment for 30 seconds. This was followed immediately by PSS perfusion for 10 minutes. Endothelium removal was confirmed by absence of vasodilatation caused by intraluminal application of acetylcholine (10^{-7}, 10^{-6} M) of segments constricted with norepinephrine. Endothelium removal was also verified at the end of the experiment by direct observation of the intimal surface after en face silver staining.

Adrenergic Nerve Activation

Two platinum wires lying 2–3 mm on either side and parallel to the axis of the artery were used for transmural electrical field stimulation. The stimulator (SD9, Grass Instruments, Quincy, Massachusetts) that drove a DC current amplifier was set to evoke 2-msec, 10-V pulses at a rate of 4–16 Hz. All constrictor responses to electrical stimulation were blocked by tetrodotoxin (10^{-7} M) or prazosin (5 x 10^{-7} M). Responses to electrical stimulation were obtained by stepwise increases in frequency after a steady-state response had occurred to the preceding frequency.

Bioassay of EDRF

A bioassay ring (1.5 cm long) denuded of endothelium was suspended on stirrups connected to a strain-gauge force transducer. The bioassay ring was superfused with PSS at a rate of 3.6 ml/min for 1 hour. During this interval, it was stretched in a stepwise manner to 7 g. The lumen of a donor carotid artery (3 cm long) with or without endothelium was perfused with PSS or dextran-PSS, and the perfusate was allowed to directly superfuse the bioassay ring. A plastic tube was also used instead of the artery as a control. The flow rate through the bioassay ring (1.5 cm long) denuded of endothelium was suspended on stirrups connected to a strain-gauge force transducer. A plastic tube was also used instead of the artery as a control. The flow rate through the bioassay ring (1.5 cm long) denuded of endothelium was suspended on stirrups connected to a strain-gauge force transducer. A plastic tube was also used instead of the artery as a control.

Shear Stress

In the experimental protocol designed to test the effects of elevated shear stress, responses to adren-ergic stimulation were compared at the same transmural pressure, during perfusion with PSS or dextran-PSS (dextran, 70,000 MW; 5% wt/vol added to PSS). The transmural pressure was estimated as the mean of the inflow and outflow pressures and was adjusted by changing the outflow resistor and inflow pressure. The viscosity of the fluids used for perfusion were measured with a kinematic viscometer (Cannon-Fenske type, Fisher Scientific, Springfield, New Jersey). At 37° C, the viscosity of PSS alone was 0.0069 poise, and dextran-PSS was 0.0203 poise. Shear stress was calculated from \( \tau = 4\eta Q/\pi r^3 \), where \( \tau \) is shear stress (dyne/cm^2), \( \eta \) is viscosity (poise; 1 poise = 1 dyne·sec/cm²), \( Q \) is flow (ml/sec), and \( r \) is radius (cm). The internal radius was calculated from the Poiseuille equation \( Q = (P_1 - P_2)nr^4/8\eta L \) where \( L \) is length (cm), and \( P_1 \) and \( P_2 \) are inflow and outflow pressures (dyne/cm²).

The resistance of the cannulae were determined by measuring flow at various perfusion pressures and linear regression lines were obtained for PSS (\( y = 0.53x - 0.4, r^2 = 98\% \)), where \( y \) is perfusion pressure and \( x \) is flow) and for dextran-PSS perfusion (\( y = 0.81x - 2.5, r^2 = 97\% \)). The regression lines were used to determine the actual inflow and outflow pressure of the vessel segment.

Drugs

The following pharmacological agents were used: acetylcholine chloride, CHAPS, dextran, methylene blue, norepinephrine bitartarate, prostaglandin \( F_{2\alpha} \), tetrodotoxin (Sigma Chemical, St. Louis, Missouri) and prazosin (Pfizer, New York, New York).

Data Analysis

In each experiment, segments from both carotid arteries of the rabbit, one with intact endothelium and the other treated with CHAPS to remove the endothelium, were used to compare responses to electrical stimulation. Vasoconstriction is expressed as vessel resistance in dyne·sec/cm² [\( R = (P_1 - P_2)/Q \times 1.332 \) where \( R \) is resistance, \( P_1 \) and \( P_2 \) are vessel inflow and outflow pressures (mm Hg), and \( Q \) is flow (ml/sec)]. Data are expressed as mean ± SEM. Statistical evaluation of the data was made with Student's t test for paired comparisons of segments from the same animal. Values of \( p < 0.05 \) were regarded as significant. In all experiments, \( n \) is the number of rabbits from which the segments were taken.

Results

Influence of Endothelium on Vasoconstriction Caused by Electrical Stimulation in Perfused Rabbit Carotid Arteries

A tracing of recordings of flow changes caused by transmural electrical stimulation (4–16 Hz) in segments with and without endothelium is shown in Figure 1. Initial flow in segments with and without endothelium was 65 ± 3 ml/min. The initial transmural pressure during perfusion with PSS was...
40.6 ± 1.5 mm Hg. The initial vessel resistance in segments with and without endothelium during perfusion with PSS were not different (7.1 ± 2.0 and 7.5 ± 2.2 \(10^3\) dyne • sec/cm\(^3\)). Vasoconstriction caused by electrical stimulation was significantly greater in segments without endothelium at all frequencies during perfusion with PSS (Figures 1 and 2). Vessel resistance during 16 Hz stimulation of segments with endothelium was 55.4 ± 6.3\% (n = 6) of the resistance of the vessel without endothelium (n = 6, p<0.05).

**Effects of Dextran**

The transmural pressure during perfusion with dextran-PSS (38 ± 2.2 mm Hg) was not significantly different from that during perfusion with PSS. The initial flow during perfusion with dextran-PSS (54 ± 3 ml/min) was significantly less than during perfusion with PSS (p<0.05). The resting vessel resistance during perfusion with dextran-PSS for segments with and without endothelium (7.1 ± 2.2 and 6.8 ± 2.2 \(10^3\) dyne • sec/cm\(^3\), respectively) was not significantly different from during perfusion with PSS. Perfusion with dextran-PSS led to significantly smaller vasoconstrictor responses in segments with endothelium when compared with responses during PSS perfusion (Figures 1 and 2). The vessel resistance during 16-Hz electrical stimulation of segments with endothelium perfused with dextran-PSS was reduced to 70 ± 7.1\% of that during perfusion with PSS (n = 6, p<0.05). The endothelium was functionally intact after perfusion of the artery with dextran as verified by the presence of vasodilation to acetylcholine (10\(^{-7}\), 10\(^{-6}\) M). Perfusion with dextran-PSS caused no significant difference in the vessel resistance during electrical stimulation of segments without endothelium (Figure 2).

**Effects of Methylene Blue**

A tracing of a recording of flow changes caused by transmural electrical stimulation (4–16 Hz) in segments with and without endothelium during perfusion with PSS or dextran-PSS in the presence of methylene blue (2 \(\times\) 10\(^{-6}\) M) is shown in Figure 3. Perfusion with methylene blue caused a significant increase in vasoconstrictor responses to electrical
stimulation in segments with endothelium only (Figures 4 and 5). For instance, during treatment with methylene blue, the resistance of vessels with and without endothelium during 4-Hz stimulation increased by 12.8±5.5 and 1.5±2.3 10^3 × dyne·sec/cm^2 during perfusion with PSS (n=6, p<0.05) and by 16.0±8.7 and 0.8±2.3 10^3 × dyne·sec/cm^2 during perfusion with dextran-PSS (n=6, p<0.05). There was no significant difference in the resistance of segments with and without endothelium during electrical stimulation during treatment with methylene blue while being perfused with PSS or dextran-PSS (Figures 4 and 5).

**Bioassay of Vasodilators Released From Endothelial Cells**

Endothelial cell-derived vasodilators were detected in a perfusion-cascade system consisting of a bioassay ring denuded of endothelium treated with atropine (5×10^-7 M) and donor segments of carotid artery with or without endothelium. Prostaglandin F_2α (PGF_2α) was used to contract the bioassay ring in the presence of atropine (5×10^-7 M). Superfusion of the bioassay ring with PSS perfusates of donor segments with endothelium caused relaxation. Perfusion with dextran-PSS caused further relaxation of the bioassay ring. PSS or dextran-PSS perfusate of donor arterial segments without endothelium caused no relaxation of the bioassay ring. Acetylcholine (ACh, 10^-6 M) perfused through the donor segment without endothelium did not cause relaxation. Nitroprusside (SNP, 10^-6 M) was added at the end of the recording to determine maximal relaxation.

**Discussion**

An isolated perfused artery was used to compare vasoconstrictor responses at the same transmural pressure and at similar initial flow rates under...
different conditions of shear stress induced by increases in viscosity. Electrical field stimulation of perfused arterial segments caused constriction of the smooth muscle that was prevented by an α1-adrenoceptor antagonist, prazosin, or blockade of neuronal conduction with tetrodotoxin. This indicates that the response of the tissue is attributable to release of norepinephrine from adrenergic nerves.

- Activation of the adrenergic nerves caused significantly greater vasoconstriction after removal of the endothelium. In isolated rings of the rabbit carotid artery, the endothelium inhibits neurogenic contractions of the smooth muscle; the inhibition of contraction is attributable primarily to the spontaneous release from the endothelium of EDRF.4

Effects of Elevated Shear Stress

Dextran added to PSS was used to increase the viscosity and thus the shear stress on the vessel wall. Increase in shear stress inhibited vasoconstriction caused by adrenergic nerve stimulation. The inhibitory influence activated by an increase in shear stress originates in endothelial cells and not from other constituents of the blood vessel wall because there was no significant effect of dextran on segments without endothelium. With a cascade-superfusion technique, the spontaneous release of endothelial cell–derived vasodilators from segments of rabbit carotid artery with endothelial cells has been demonstrated to inhibit smooth muscle contraction.4 With a similar technique, increase in perfusate flow from 2 to 4 ml/min was demonstrated to increase release of vasodilator from endothelium of isolated dog femoral artery.9 In the present study, increasing endothelial cell shear stress by perfusing donor segments with dextran at constant flow caused further relaxation of a bioassay ring, suggesting an augmented release of vasodilator. It is, therefore, likely that vasoconstrictor responses to nerve stimulation were inhibited during an increase in shear stress attributable to a greater release of endothelium-derived vasodilators.

- Because the transmural pressure determines the initial smooth muscle cell length, the effects of the high and low shear stress on the vessel were compared at the same initial transmural pressure. Maintaining transmural pressure constant allowed comparisons of vasoconstrictor responses of the blood vessels under high and low shear stress. While it is true that the normal perfusion pressure of the rabbit carotid artery in vivo is likely to be more than 40 mm Hg, this pressure was chosen because it yielded maximal vasoconstrictor responses. The effects of increased shear stress observed in this study were still demonstrable in this preparation at higher perfusion pressures (data not shown), but because the vasoconstriction was less, the changes induced by dextran were of smaller magnitude.

Flow was decreased during perfusion with dextran by approximately 17%. Because vessel resistance was not changed, this decrease in flow was not likely due to a change in diameter of the blood vessel. Furthermore, there was no difference in the resting diameter of the blood vessel during perfusion with PSS or dextran solution (0.08 and 0.09 cm, respectively, as calculated from Poiseille's equation). The cannulae resistances were higher during dextran perfusion compared with PSS perfusion as reflected by the higher slope of the pressure-flow calibration curves. Thus, the decrease in flow was due to the increased resistance of the perfusion cannulae during dextran perfusion.

- With values of flow and calculated radius, the calculated initial shear stress during perfusion with PSS was $171.3 \pm 17.7$ dyne/cm$^2$ and with dextran-PSS it was $248.3 \pm 28.7$ dyne/cm$^2$. Although initial shear stress was increased by raising the viscosity of the perfusate, shear stress must have increased further as a result of the changes in diameter during vasoconstriction. For instance, vessels with endothelium perfused with control solution constricted in response to 16 Hz to a calculated diameter of 0.05 cm, while those perfused with dextran constricted to 0.07 cm. Although vessels with endothelium constricted less during dextran perfusion, the shear stress remained larger during dextran perfusion than during perfusion with control solution. The calculated shear stress during vasoconstriction caused by 16-Hz stimulation during dextran perfusion was 349 dyne/cm$^2$ compared with 304 dyne/cm$^2$ during perfusion with control solution. Thus, shear stress remained greater during perfusion with dextran during vasoconstriction as well as at rest.

The action of dextran was mediated by its effects on viscosity of PSS. The lack of nonspecific effects of dextran is suggested by the fact that it had no significant effect on segments without endothelium. Furthermore, dextran added to isolated rings of rabbit carotid artery with endothelium in organ chambers did not affect baseline isometric tension or norepinephrine-induced tone (unpublished observations). These observations indicate that dextran had no direct effects on the endothelium, independent of its effect on viscosity of the solution. In addition, the contraction caused by electrical stimulation of rings of artery with and without endothelium were unchanged by dextran, indicating the lack of nonspecific effects on adrenergic nerve stimulation. Thus, for instance, it is unlikely that the higher perfusate viscosity decreased diffusion of endothelial cell–derived vasodilators into the lumen, thereby inhibiting vasoconstriction. As observed for isolated rings of artery,4 ascorbate ($10^{-4}$ M), a free radical scavenger, had no effect on the vasoconstriction caused by electrical stimulation during perfusion with PSS or dextran-PSS, indicating that free radicals generated during electrical stimulation did not contribute to any of the effects observed.

An increase in shear stress has been shown to activate endothelial cell histidine decarboxylase in the rabbit aorta.10 Histamine constricts the rabbit carotid artery (unpublished observations), making it
unlikely that released histamine acting on carotid artery smooth muscle could explain the augmented inhibitory influence exerted by dextran.

**Effect of Inhibition of Guanylate Cyclase**

The nature of EDRF has not yet been established, but endothelium-dependent relaxation is associated with a rise in cyclic guanosine monophosphate content of the smooth muscle cells,17–19 which may be prevented by methylene blue.18,20 In this study, methylene blue increased vasoconstriction caused by electrical stimulation in endothelium containing segments only, while no change was observed in denuded segments. Methylene blue abolished the difference in vasoconstriction of segments with and without endothelium during perfusion with PSS or dextran. Thus, it is likely that the endothelium exerts its inhibitory influence on neurogenic vasoconstriction in the perfused rabbit carotid artery by way of guanylate cyclase, which is activated by EDRF.19–21 That dextran had no effect in the presence of methylene blue suggests that increased shear stress may exert at least part of its effect by EDRF. It is likely that EDRF inhibits neurogenic vasoconstriction primarily by acting directly on smooth muscle as demonstrated in the bioassay experiments. Increases in flow have been shown to enhance release of prostacyclin from endothelial cells in culture and from the endothelium of perfused arteries.9,10 It is possible that prostacyclin release could explain part of the action of dextran, although this is made less likely by the action of methylene blue. It has also been reported that prostacyclin does not account for flow-induced, presumed shear stress-mediated vasodilation of canine coronary arteries in vivo or in vitro.6,9

**Endothelial Cell Shear Stress and Adrenergic Vasoconstriction**

Experiments in vivo have demonstrated that increases in blood flow dilate large blood vessels including the coronary and femoral arteries.3–8 In agreement with these earlier observations, the present study suggests that increases in shear stress inhibit adrenergic neurogenic vasoconstriction of a large artery by augmenting release of endothelial cell vasodilators. In previous studies, increased shear stress induced by dextran was shown to mediate an endothelium-dependent decrease in vasoconstriction of isolated perfused mesenteric resistance arteries induced by exogenously applied norepinephrine.13 This may indicate that sympathetic vasoconstriction of large and small arteries might be modulated by blood flow and augmented if the endothelium is dysfunctional because of disease.

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