Shear Stress–Induced Release of Prostaglandin H₂ in Arterioles of Hypertensive Rats

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Abstract—The nitric oxide–mediated portion of shear stress–induced dilation of rat gracilis muscle arterioles was shown to be impaired in spontaneously hypertensive rats (SHR). Because shear stress–induced dilation is primarily mediated by endothelium-derived prostaglandins in rat cremasteric arterioles, we hypothesized that in the cremasteric vascular bed the mediation of shear stress–induced dilation by prostaglandins is altered in hypertension. At a constant intraluminal pressure of 80 mm Hg, the active diameters of isolated rat cremasteric arterioles of normotensive 30-week-old Wistar-Kyoto rats (WKY) and SHR were 58.0±3.1 and 51.7±3.6 μm, respectively, whereas their passive diameters were 109.4±4.4 and 101.9±6.7 μm, respectively. Dilations to increases in shear stress elicited by increases in intraluminal flow (from 0 to 25 μL/min) were significantly less (P<0.05) in cremasteric arterioles isolated from SHR than from WKY. Arachidonic acid (10⁻³ mol/L) elicited constrictions in SHR arterioles but dilations in WKY arterioles. The prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂) receptor antagonist SQ 29,548 (10⁻⁶ mol/L) significantly increased basal diameter by 11% and normalized the attenuated shear stress–induced dilation in SHR, whereas it did not affect basal diameter and arteriolar responses of WKY. Furegrelate, a specific inhibitor of TxA₂ synthase, did not affect the response in SHR. Also, SQ 29,548 reversed the arachidonic acid–induced constriction to dilation in SHR arterioles, whereas it did not affect the dilator response in WKY arterioles. Constrictions of arterioles of WKY and SHR to U46,619 (a PGH₂/TxA₂ receptor agonist) were not different. These results demonstrate that in cremasteric arterioles of hypertensive rats, shear stress elicits an enhanced release of PGH₂, resulting in a reduced shear stress–dependent dilation. Thus, augmented hemodynamic forces can alter the shear stress–induced synthesis of prostaglandins, which may contribute to the elevated vascular resistance in hypertension. (Hypertension. 2000;35:925-930.)

Key Words: hypertension, genetic ■ microcirculation ■ muscle, cremasteric ■ prostaglandins

In vivo studies of the skeletal muscle microcirculation suggest that a reduction in the caliber and number of vessels¹–⁴ contributes to the increased peripheral resistance in hypertension. However, it is likely that functional changes occur before an alteration in the structure of microvessels. Previous studies have indicated that the endothelium plays a pivotal role in the regulation of arteriolar diameter via the production and release of dilator factors⁵ to a variety of agonists. In vivo, the primary stimulus for the release of endothelial factors is a change in wall shear stress (WSS), which accompanies changes in flow velocity or viscosity of the blood.⁶ It has been shown that in response to increases in flow/WSS, both nitric oxide (NO) and prostaglandins are produced in the endothelium of rat gracilis muscle arterioles; thus, they are importantly involved in the modulation of arteriolar resistance.

Previously, it has been found that the shear stress–dependent dilation of gracilis skeletal muscle arterioles is significantly reduced in spontaneously hypertensive rats (SHR) compared with normotensive rats.⁸,⁹ This is primarily due to the absence of the NO-mediated portion of the response.⁸ We have also shown that in isolated gracilis arterioles, an acute increase of intraluminal pressure impairs the NO-mediated portion of shear stress–induced dilation because of an increased superoxide production.¹⁰ These findings suggest that high pressure may interfere specifically with the shear stress–induced release/synthesis of NO, most likely via an increased release of reactive oxygen species.¹¹ Yet, there are studies showing that hypertension elicits an enhanced production of prostaglandin H₂ (PGH₂) in large vessels.¹²–¹⁵ Recent studies in microvessels suggest that an alteration in the production and/or release of endothelium-derived constrictor factors, such as PGH₂/thromboxane A₂ (TxA₂), could, in addition to the lack of NO, also account for the increased vascular resistance in various forms of hypertension.¹⁷–¹⁹

Previously, we have found that shear stress–dependent dilation in arterioles of rat cremaster muscle of normotensive rats is mediated solely by endothelium-derived dilator prostaglandins,²⁰,²¹ a condition that allows us to test the idea whether this prostaglandin-dependent response is compromised in genetically hypertensive rats. In this context, we
previously found an enhanced release of PGH₂ to pressure and vasoactive substances in these vessels. On the basis of the aforementioned studies, we hypothesized that shear stress–induced dilation is reduced because of the altered metabolism of prostaglandins. To test this hypothesis, we investigated the changes in diameter of isolated cremaster arterioles of normotensive and SHR as a function of WSS (with constant intravascular pressure in the vessels) in the absence and presence of a PGH₂/TXA₂ receptor antagonist or a TXA₂ synthase inhibitor.

Methods

The studies were conducted on isolated arterioles (~55 μm in diameter) of cremaster muscle of 30-week-old male normotensive Wistar-Kyoto rats (WKY) and SHR. All experimental protocols were performed in accordance with institutional guidelines. Systolic blood pressure of conscious rats was measured by the tail-cuff method. Rats were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal, 50 mg/kg). The isolation procedure of cremaster muscle arterioles has been described previously. Briefly, the cremaster muscle of rats was exposed by an incision of the skin. The muscle then was cut out and placed on a Petri dish containing cold (0°C to 4°C) salt solution (pH 7.4), which was composed of (mmol/L) NaCl 145, KCl 5.0, CaCl₂ 2.0, MgSO₄ 1.0, NaH₂PO₄ 1.0, dextrose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS 3.0. Rats were euthanized by an overdose of Nembutal.

A segment (~1 mm in length) of an arteriole, branching off from the main arteriole supplying the muscle, was isolated from the cremaster muscle and surrounding tissue and transferred to the vessel chamber. The physiological salt (PS) solution used for suffusion and perfusion of the vessels contained (mmol/L) NaCl 110.0, KCl 5.0, CaCl₂ 2.5, MgSO₄ 1.0, dextrose 10.0, NaHCO₃ 24.0, and EDTA 0.02 and was equilibrated with a gas mixture of 21% O₂, 5% CO₂, balanced with N₂, at pH 7.4 (34°C). From a reservoir (60 mL), the vessel chamber (15 mL) was continuously supplied with PS solution at a rate of 40 μL/min. After the vessel was mounted on the proximal pipette, the perfusion pressure was raised to 20 mm Hg to clear the debris from the lumen. Then, the other end of the vessel was cannulated, the system was perfused for several minutes. Then, the perfusion pressure was slowly increased to 80 mm Hg. The temperature was set to 34°C (YSI temperature controller), and the vessels were equilibrated to a new diameter for ~1 hour.

Experimental Procedure

Vessels were allowed to develop spontaneous tone in response to intraluminal pressure in the absence of vasoactive agents. After the equilibration period, the vessels were exposed to increases in perfusate flow from 0 to 25 μL/min in 5-μL/min steps. Flow was established at a constant intravascular pressure (80 mm Hg) by changing proximal and distal pressures to an equal degree, but in opposite directions, to keep midpoint luminal pressure constant. Responses to vasoactive agents were tested at 80 mm Hg perfusion pressure in no-flow conditions. All drugs were added to the reservoir connected to the vessel chamber, and final concentrations are reported. After responses to each drug subsided, the vessel chamber was flushed with PS solution. At the conclusion of each experiment, the suffusion solution was changed to a Ca²⁺-free PS solution that contained EGTA (1.0 mmol/L) to assess the level of active tone generated by the arterioles in response to intravascular pressure. The vessels were incubated for 10 minutes, and then the passive diameter of arterioles at 80 mm Hg perfusion pressure was obtained.

The role of constrictor prostaglandins in shear stress–induced dilation of cremaster muscle arterioles was studied in the following manner: After obtaining control responses, the PGH₂/TXA₂ receptor antagonist SQ 29,548 (10⁻⁶ mol/L) was added to the suffusion solution. In arterioles of SHR, the effect of furegrelate (5×10⁻⁶ mol/L), a TXA₂ synthase inhibitor, on shear stress–induced dilation was tested. After an incubation period (~30 minutes), the shear stress–diameter relations were again assessed. Also, arteriolar responses to arachidonic acid (AA, 10⁻⁴ mol/L), prostaglandin E₂ (PGE₂, 10⁻⁴ mol/L), and U46,619 (10⁻⁶ mol/L), a stable PGH₂/TXA₂ receptor agonist, were obtained before and after the vessels were exposed to SQ 29,548.

All salts and chemicals were obtained from Sigma Chemical Co or Cayman Chemical Co. SQ 29,548, furegrelate, AA, PGE₂, and U46,619 were dissolved in ethanol as stock solutions and were diluted with PS solution. The diameters of vessels and peak responses were measured with an image-shearing monitor (model 907, IPM) and recorded with an X-Y recorder (model MC6625, Multitron). The flow was measured by a ball flowmeter (Omega Engineering Inc), which was calibrated by a perfusion pump (Harvard Apparatus Co, Inc) in which flow rate was accurate in the range of 0 to 100 μL/min. WSS was calculated from diameter (2r) and flow data according to the following equation: \( WSS = 4\pi Q/(\pi r^4) \), where \( Q \) is the flow rate and \( r \) is the vessel radius. Relationships between shear stress and diameter were obtained for arterioles of both strains of rats in control conditions and during the use of SQ 29,548 or furegrelate. Changes in diameter in response to vasoactive agents were normalized to the corresponding passive diameter and expressed as percent changes. Data are mean±SEM; n refers to the number of rats. Statistical analyses were performed by ANOVA, followed by Tukey post hoc test, regression analysis, and paired and grouped Student t tests, as appropriate. A value of \( P<0.05 \) was considered significant.

Results

The systolic blood pressure of the SHR (212.0±8.2 mm Hg, n = 7) was significantly (\( P<0.05 \)) higher than that of the WKY rats (127.1±4.9 mm Hg, n = 7). The active diameters of arterioles of WKY and SHR (obtained in the presence of constant intravascular pressure of 80 mm Hg and static flow conditions) were significantly different at 58.0±3.1 and 51.7±3.6 μm, respectively, whereas their corresponding passive diameters were 109.4±24 and 101.9±9 μm. Normalizing the active diameter to their corresponding passive diameter indicates no significant difference in basal tone of arterioles (~50%).

The data related to diameter changes of arterioles of WKY and SHR as a function of WSS under control conditions are summarized in the top panel of Figure 1. The nearly vertical increase of the shear stress–diameter curve at ~35 dyn/cm² demonstrates the marked sensitivity of normotensive arterioles to increases in shear stress, to which they respond with substantial dilation. In contrast, the diameters of hypertensive arterioles did not increase at all in this range of shear stress, and only at >80 dyn/cm² did diameters increase significantly. The significant difference in the slopes of the shear stress–diameter curves indicates that arterioles from SHR dilate to a lesser degree in response to increases in shear stress. Also, as shown in the top panel of Figure 1, the maximal increase in diameter was significantly less in arterioles of SHR (17±2.2 μm) than in arterioles of WKY (27.3±4.1 μm). The bottom panel of Figure 1 shows that for a given intra-arteriolar flow, WSS is significantly higher in vessels of SHR compared with WKY.

To examine whether constrictor prostaglandins are involved in the shear stress–induced response, we used 10⁻⁶ mol/L SQ 29,548, a PGH₂/TXA₂ receptor blocker, after
obtaining control responses. In normotensive rats, SQ 29,548 did not significantly affect shear stress–induced arteriolar dilation (Figure 2, top) and basal arteriolar tone. In contrast, in cremasteric arterioles of SHR, SQ 29,548 significantly increased basal diameter (to 57.3±3.5 μm) and augmented the arteriolar dilation in response to increases in shear stress (Figure 2, middle). In separate experiments, we found that furegrelate (5×10⁻⁶ mol/L) did not affect shear stress–induced responses of arterioles from SHR (Figure 2, bottom). Comparison of shear stress–normalized diameter curves of arterioles of WKY in control conditions and SHR in the presence of SQ 29,548 revealed no significant difference between the slopes of the curves (Figure 3).

AA (10⁻⁵ mol/L), the precursor of prostaglandins, elicited dilation of arterioles of WKY, whereas it constricted arterioles of SHR. Also, responses to PGE₂ (10⁻⁵ mol/L) were significantly attenuated in SHR compared with WKY (Figure 4), whereas constrictor responses to the PGH₂/TxA₂ receptor agonist U46,619 were not different in arterioles of the 2 strains of rats (Figure 4). Incubation of arterioles with SQ 29,548 completely abolished constrictions to U46,619 and reversed the AA-induced constriction to dilation in SHR, whereas it did not affect the responses of WKY arterioles. Also, in the presence of SQ 29,548, dilations of WKY and SHR arterioles to PGE₂ were similar.

**Discussion**

The salient finding of the present study is that shear stress–induced dilation of cremasteric arterioles of SHR is significantly reduced compared to that of vessels of normotensive rats and that this reduced arteriolar response is primarily due to the enhanced synthesis of PGH₂.
Previous investigations of the microcirculation of hypertensive animals has revealed morphological changes in the vascular wall as well as changes in the structure of the arteriolar network. Recent studies have suggested that an altered function of arteriolar endothelial cells is also involved in the development and/or maintenance of increased arteriolar resistance in hypertension. Studies of ring preparations of aorta and mesenteric arteries of hypertensive rats and studies in hypertensive humans have indicated that the endothelial synthesis of NO and perhaps other endothelial mechanisms of peripheral vessels could be impaired. In vivo and in vitro studies have demonstrated that the endothelium contributes to circulatory homeostasis by a shear stress–dependent regulation of vascular resistance that is important primarily in microvessels. Increases in WSS can take place during increases in blood flow velocity, a condition that is likely to be present in hypertension, because a higher blood pressure drop and reduced vessel diameters are present concurrently. Previous studies have revealed that the flow/shear stress–sensitive dilation of arterioles in certain tissues and organs is reduced in SHR and Dahl salt-sensitive rats and that the dysfunction of this mechanism relates primarily to the lack of NO mediation. Interestingly, in a recent study, Izzard and Heagerty showed a reduced flow-dependent dilation in small mesenteric arteries of SHR and suggested that an impairment in the NO pathway cannot account for this observation. The alterations in the arteriolar synthesis/release of prostaglandins in response to changes in WSS in hypertension is less known. In the present study, we have used cremasteric arterioles because they release only prostaglandins in response to increases in shear stress, thus allowing us to test the hypothesis that high hemodynamic forces interfere with the synthesis and release of prostaglandins as well.

Attenuation of Shear Stress–Induced Dilation in Hypertension

In response to increases in shear stress, isolated cremasteric arterioles exhibited a greatly reduced dilation in hypertensive rats compared with normotensive rats, as indicated by the significant right shift in the slope of the shear stress–diameter curve of arterioles of SHR compared with WKY (Figure 1, top). The importance of this finding is further underscored by the fact that for a given flow, significantly higher shear stress develops in vessels of SHR (Figure 1, bottom). This indicates the significant impact of increased resistance to blood flow that results in a greater power dissipation in hypertension and may also be partly responsible for functional rarefaction and remodeling of the vascular wall observed in cremaster muscle microcirculation in hypertension. Previous studies have shown that shear stress–dependent dilation of cremaster muscle arterioles is mediated primarily by endothelium–derived prostaglandins, inasmuch as indomethacin completely eliminated flow- and viscosity-induced dilation both in vivo and in vitro. Thus, we hypothesized that alterations in the mediation of the response by prostaglandins might be responsible for the observed reduction in shear stress–induced dilation in this vascular bed.

Possible Role of Constriction Prostaglandins

In normotensive rats, inhibition of PGH$_2$/TxA$_2$ receptors did not significantly affect the cremasteric arteriolar dilation to increases in WSS, suggesting no role for PGH$_2$/TxA$_2$ in the mediation of shear stress–induced arteriolar responses in normotension. In contrast, shear stress–induced dilation in cremasteric arterioles from hypertensive rats is markedly enhanced in the presence of SQ 29,548 (Figure 2). Also, in the presence of SQ 29,548, shear stress–induced dilations of arterioles from WKY and SHR were not different (Figure 3).
These findings suggest that increases in shear stress elicit an enhanced release of PGH₂/TxA₂ in cremasteric arterioles of SHR that counteracts the dilation. The nature of the constrictor prostaglandin was further investigated by use of furegrelate, a specific blocker of TxA₂ synthase. Because furegrelate did not affect the shear stress–diameter relation, it is likely that increased synthesis and/or accumulation of PGH₂ is responsible for the reduced dilator response to shear stress in SHR.

In the present study, it was also shown that AA elicits dilation of WKY arterioles but constriction of SHR arterioles. The finding indicates that the metabolism of AA is altered in SHR arterioles. The reversal of AA-induced constriction to dilation by SQ 29,548, a PGH₂/TxA₂ agonist, were not different in the 2 strains, arguing against the possibility that an enhanced density of PGH₂/TxA₂ receptors is responsible for the alteration in flow-dependent response in hypertension.

Our previous study revealed that at an early age, flow-dependent dilation is still present in arterioles of SHR, suggesting that the prevailing hemodynamic conditions (eg, increased flow velocity and/or pressure) to which these arterioles are exposed as hypertension develops are causing the impairment in endothelial function. One reason for this change in hypertension could be an alteration in the “rheoreceptors” or the endothelial signaling pathway that links the increase in shear stress to the release of AA from the plasma membrane when the endothelium is chronically exposed to high intraluminal hemodynamic forces, such as pressure and/or shear stress. The increased level of AA then is converted by cyclooxygenase-1 and/or -2 to PGH₂. There are studies suggesting that in hypertension there is an increased release of NO that, however, is accompanied by an increased release of superoxide. These 2 substances can form peroxynitrite, a free radical, which has been shown to inhibit prostaglandin I₂ synthase. Moreover, PGH synthase can generate superoxide, and PGH₂ can interact with NO. These interactions may be responsible for the inability of endothelium to metabolize PGH₂, produced in response to increases in shear stress, which results in an excess amount of PGH₂. The idea that the unmetabolized PGH₂ is responsible for the impaired shear stress–dependent dilation is further supported by our previous findings that enhanced myogenic constriction in hypertension could be inhibited by SQ 29,548, but not by TxA₂ synthase, blockade. Previously, we found that in addition to PGH₂, endothelin also contributes to the enhanced myogenic response of arterioles in hypertension. Release of constrictor factors to shear stress is not unique to cremasteric arterioles. In skeletal muscle venules after blocking the synthesis of NO and prostaglandins, flow elicits an endothelin-dependent constriction. In small mesenteric arteries of hypertensive rats, endothelin is released in response to flow, and if SHR are treated with an endothelin receptor blocker, then dilation to flow is augmented. The pathophysiological role of PGH₂ in this process is underscored by studies of Iwama et al showing that the production of PGH₂ correlates well with the level of blood pressure in SHR. The inability of the inhibition of PGH₂/TxA₂ receptors to elicit marked reduction in blood pressure in hypertension indicates that other endothelial and nonendothelial mechanisms may be altered as well.

In conclusion, the present study demonstrates a reduced shear stress–induced dilation of cremasteric arterioles of genetically hypertensive rats. The impaired dilation is due to an enhanced synthesis and/or accumulation of PGH₂ in response to shear stress. Thus, the present findings suggest an important role for the enhanced synthesis of constrictor prostanoids in the regulation of vascular resistance by shear stress in hypertension.

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References


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