In Vivo Platelet Thrombus Formation in Microvessels of Spontaneously Hypertensive Rats

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Sustained high blood pressure causes functional changes in both vascular endothelial cells and platelets. Therefore, we hypothesized that in vivo platelet thrombus formation would be increased in the cremaster muscle microvessels of rats during genetic hypertension. Experiments were carried out on spontaneously hypertensive rats (SHR) and normotensive Wistar Kyoto rats (WKY) at 12 weeks of age. Fluorescein isothiocyanate tagged to bovine serum albumin (FITC-BSA) was injected intraarterially and 450 to 490 nm light was used to activate the FITC-BSA and induce a thrombus within the vasculature. In vivo television microscopy was used to quantitate thrombus formation and microvascular diameter changes. The time of platelet thrombus initiation and subsequent time of thrombus growth were studied at wall shear rates of approximately 2000 sec^{-1} and 270 sec^{-1} in third-order arterioles and venules, respectively. In SHR, times for platelet thrombus initiation and vessel occlusion were significantly less in both arterioles and venules, whereas time for platelet thrombus growth following initiation was significantly prolonged. Greater shear rates in arterioles compared to venules decreased platelet adhesion and subsequently decreased the rate of thrombus formation in both WKY and SHR groups. However, the ratio of WKY to SHR platelet thrombus growth (platelet aggregation) time remained similar (0.83 ± 0.06 in arterioles and 0.79 ± 0.06 in venules). These results indicate that there is increased thrombus formation during hypertension and that the platelet adhesion processes may be of greater importance than platelet aggregation in producing this increase. Am J Hypertens 1997;10:1140–1146

KEY WORDS: **Hematocrit, microvessels, platelets, spontaneously hypertensive rat, Wistar-Kyoto rat, thrombus formation, viscosity.**

Basic science and clinical research indicate that hypertension is often characterized by changes in blood vessels and in blood rheological properties, which have major roles in initiating and in maintaining an elevation in blood pressure. Different alterations to different components of the blood thrombotic processes have been observed in hypertensive disease. These include increased whole blood viscosity at low (<208 sec^{-1}) shear rates,[1,2] increased plasma viscosity,[1,2] enhanced plasma proteins vWF,[3] and particularly fibrinogen[4] concentrations, which are the major determinants of

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plasma viscosity. Hematocrit[2] and erythrocyte[2,5,6] and platelet[7,8] aggregation indices are also increased during hypertension.

Changes in the properties of platelets and endothelial cells and their interactions appear to have a major influence on thrombogenesis during the hypertension development. Moreover, there is evidence that these abnormalities of rheological and thrombotic properties occur in hypertensive patients even before they sustain any vascular complications.[9]

In vitro aggregometry has shown that platelet aggregation to ADP is either increased[10] or unchanged[11] for hypertensive patients. However, we are not aware of any direct in vivo studies of platelet adhesion or aggregation in humans.

Similar to the clinical studies with humans, there are no reported in vivo studies involving the direct observation of platelet thrombus formation in the resistance vessels of spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats. Because erythrocyte and platelet aggregation indices are increased in hypertension[2,5–8] and production of the vasodilator/antiaggregatory agent nitric oxide (NO) is depressed in the SHR model,[12] we hypothesized that thrombogenesis would be increased in SHR in vivo.

The aim of the current research was to determine changes that occur in the platelet adhesion and platelet aggregation processes during genetic hypertension of thrombogenesis. In vivo platelet thrombus formation during increased chronic arterial blood pressure was studied in the SHR cremaster muscle microcirculation. Comparisons were made between acute focal injuries to individual microvessels in the hypertensive SHR and the normotensive WKY rats. The phenomena were studied by direct observations of the microcirculation by using intravital video microscopy and by measurements of blood flow parameters and vessel diameters, which could influence platelet thrombus formation. Hematocrit and blood and plasma viscosity were also determined.

METHODS

Animals and Blood Collection Male SHR and WKY rats were purchased from Charles River Laboratories Inc. (Wilmington, MA) at 12 weeks of age. Rats were anesthetized with sodium pentobarbital (50 mg/kg; intraperitoneally), and blood was withdrawn by venipuncture of the vena cava using a 19-gauge needle and polypropylene syringes containing sodium citrate anticoagulant (10.9 mmol/L) with a ratio of 1 part anticoagulant to 9 parts blood. To obtain plasma, the blood was centrifuged at 2000 *g* for 15 min at room temperature. For hematocrit, platelet count, and blood viscosity measurements, 0.6 mL of blood was withdrawn into the syringes, which were previously rinsed with sodium EDTA (54 mmol/L EDTA in 0.85% NaCl, pH 7.4). Microhematocrit was also determined for each (n = 8 in WKY and n = 7 in SHR group) animal using a microhematocrit centrifuge. The number of platelets in blood from the WKY (n = 7) and SHR (n = 6) groups was counted in a hemocytometer.

Blood and Plasma Viscosity Measurements For measurement of blood and plasma viscosity (n = 8 in WKY and n = 7 in SHR group), Brookfield's ''cone and plate'' type viscometer was used (Model LVDV-II, with cone angle 0.8°, Brookfield Engineering Laboratories, Inc., Stoughton, MA). Blood (η_B) and plasma (η_P) viscosity were measured at shear rates of 75, 90, 150, and 225 sec^{-1} at 26 to 27°C. However, because the plasma viscosity is shear rate independent,[13] the obtained results were averaged and used to determine relative blood viscosity. Relative blood viscosity (η_B/η_P) indicates the contribution of the red blood cells to blood viscosity independent of the plasma viscosity effect.

In Vivo Platelet Thrombus Formation In preparation for in vivo experimentation, the rats were anesthetized with sodium pentobarbital (50 mg/kg; intraperitoneally) and had a tracheal cannula inserted to maintain a patent airway. A carotid artery cannula was used to continuously monitor mean arterial blood pressure and heart rate with a transducer and a MicroMed Blood Pressure Analyzer (Louisville, KY). The skin of the right side of the scrotum was opened and the cremaster was incised longitudinally, keeping the principal nerves and blood vessels to the muscle intact. The cremaster was spread with sutures over the glass slide, moistened with modified Krebs solution (113 mmol/L NaCl, 2.5 mmol/L $NaHCO_3$, 11.6 mmol/L dextrose, 4.7 mM KCL, 2.6 mmol/L $CaCl_2 \cdot 2H_2O$, 1.2 mmol/L $MgSO_4 \cdot 7H_2O$, and 1.2 mmol/L KH_3PO_4), and covered by a cellophane sheet. Animals were placed on a heating pad to maintain rectal temperature at 36° to 37°C.

The animal and cremaster preparation were positioned on a modified stage of a microscope (Nikon, Tokyo, Japan) so that the cremaster, which is approximately 200 to 250 μm thick, could be observed by transmitted light or epi-illumination. A closed circuit television system was used to view the platelet thrombus formation in the microcirculation.

Third-order arterioles (3A) and third-order venules (3V) were chosen for observation using a branching nomenclature. The single largest arteriole that supplies blood to the cremaster muscle was termed the first-order arteriole (1A). Branches of the 1A were termed second-order arterioles (2A), and their branches were termed third-order arterioles (3A). Similar ordering was chosen for venules.

Following the surgical preparation and preceding each experiment, there was a 30-min equilibration pe-

riod. After the equilibration, fluorescein isothiocyanate tagged to bovine serum albumin (FITC-BSA, 0.2 ml/100g of body wt) was injected intraarterially and allowed to circulate for 20 min. Epi-illumination with blue light (450 to 490 nm) from a mercury arc lamp[14] was used to first photoactivate a segment of a third-order arteriole (24 to 28 mm in diameter) and then a segment of a third-order venule (24 to 32 mm in diameter) in both SHR (n = 6) and WKY (n = 7) rats. This technique causes microvessel platelet thrombus formation and hemostasis without endothelial denudation and exposure of the subendothelium.[15,16] The power density (set at 0.018 W/mm^2) was measured at the focal plane of the objective by using an optometer (model 1815, Newport/Klinger, Irvine, CA).

Blood Flow Velocity Measurements and Wall Shear Rate Quantification Mean blood flow velocity was measured using an IPM RBC velocity tracker (Model 102B-C, San Diego, CA) and values of shear rates were calculated during the experiments. The measurements of blood flow velocity were made by using the dual sensor cross-correlation technique,[17] which has been widely used to estimate blood flow in vivo as well as in vitro. In the present study we used the Pittman and Ellsworth[18] method for quantification of the conversion factor for mean blood flow velocity calculation. This method is based on the ratio of the size of the sensing region to the observed vessel diameter and provides very accurate calculations of blood flow velocity.[18] Therefore, on the assumption of a parabolic profile of blood flow velocity as a first approximation, the wall shear rate ($\dot{\gamma}$) was quantified from the following equation using the blood flow centerline velocity V, and the Pittman-Ellsworth's correction factor k[18] as:

$$\dot{\gamma} = 8V/kD$$

where D is the internal vessel diameter.

Prior to photoactivation in each vessel, wall shear rate was measured and then adjusted if necessary to give a desired wall shear rate of approximately 2000 sec^{-1} in arterioles and 270 sec^{-1} in venules. Any adjustment of shear rate was done by a partial manual restriction of the appropriate second-order arterioles or venules with a polished glass pipet and a micromanipulator was used if necessary. Photoactivation was not started until wall shear rate in the selected vessel remained unchanged for at least 5 min. The image of the cremaster muscle microvessel was displayed on a calibrated monitor and the red blood cell column and luminal diameter of vessel were measured from a television monitor screen at 5-min intervals. Photoactivation continued until complete vessel occlusion was determined. The vessel was considered to be completely occluded when the flow stopped for at least 30 sec.

Calculations of Rate of Vessel Occlusion Three major variables were used to quantitate the platelet thrombus formation process: time for platelet thrombus initiation (adhesion), time for platelet thrombus growth following thrombus initiation (aggregation), and shear rate change. The time of platelet thrombus initiation is the time period from the start of photoactivation until a visible platelet thrombus appeared. The time period for platelet thrombus growth following platelet thrombus initiation was expressed as a percent of full time period necessary for complete vessel occlusion.

Assuming that the value of wall shear rate was changing from its initial maximal value (prior to photoactivation) to zero (occluded vessel) during the time period necessary for complete vessel occlusion, we calculated the variable ''shear rate change,'' which represents changes of wall shear rate during the time period necessary for vessel occlusion. It is expressed in sec^{-2}:

$$\text{Shear rate change} = \frac{\Delta \text{ wall shear rate} - \dot{\gamma}}{\Delta \text{ vessel occlusion time} - t}$$

$$= \frac{[sec^{-1}]}{[sec]} = [sec^{-2}]$$

We have calculated the ratio of all variables (platelet thrombus initiation time, growth period, and rate of vessel occlusion) for WKY rats to SHR rats at all wall shear rates used in both arterioles and venules. This was done to determine whether the effects of shear rate or abnormalities caused by hypertension development are the result of changes in platelet-to-endothelial cell (thrombus growth period to platelet aggregation) or thrombus formation in general (shear rate change).

After the photoactivation of both an arteriole and a venule, a second technique for inducing a focal, acute vessel injury was used. A glass micropipet and a micromanipulator were used to puncture a second-order venule (60 to 80 μm in diameter). This technique mechanically disrupts the entire vascular wall and permits adhesion of the circulating platelets to the subendothelial collagen.[19] Bleeding time was then measured as an index of platelet plugging and hemostasis.

Statistical Analysis Values given in the text and figures are means ± SEM. Compared data were evaluated by Student's unpaired t test. Differences between groups were considered statistically significant if $P < .05$.

RESULTS

Comparisons of SHR and WKY rats did not demonstrate any significant difference in body weight, whereas both mean arterial blood pressure and heart

TABLE 1. COMPARISONS OF BODY WEIGHT, MEAN ARTERIAL BLOOD PRESSURE, HEART RATE, HEMATOCRIT, AND PLATELET COUNT IN NORMOTENSIVE WISTAR KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

	WKY (n = 7)	SHR (n = 6)
Body weight (g)	299 ± 13	296 ± 10
Mean arterial blood pressure (mm Hg)	103 ± 5	151 ± 4*
Heart rate (beats/min)	365 ± 15	407 ± 9*
Hematocrit (%)	42 ± 1	47 ± 2*
Platelet count × $10^4/\mu$L blood	906 ± 25	1197 ± 30*

All values are mean ± SEM.

** Significantly different compared to WKY group values for* $P < .05$.

rate were significantly higher in spontaneously hypertensive rats (Table 1). Hematocrit and the number of circulating platelets were also increased in SHRs compared to WKY rats (Table 1).

Plasma viscosity was significantly higher in the hypertensive animals than in the WKY group (Table 2). Blood viscosity was also increased in SHRs as compared to control animals (Table 2), and the extent of this elevation was inversely related to shear rate. At a relatively higher shear rate (225 sec^{-1}), difference in blood viscosity was approximately 89% higher in hypertensive animals, whereas at the lower shear rate (75 sec^{-1}), the difference was 123% (Table 2). The relative viscosity of blood (η_B/η_P) was also significantly elevated in the SHR group compared to the WKY group (Table 2).

TABLE 2. COMPARISONS OF PLASMA AND ABSOLUTE AND RELATIVE BLOOD VISCOSITIES IN NORMOTENSIVE WISTAR KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

	WKY (n = 8)	SHR (n = 7)
Plasma viscosity (mPa·sec)	1.41 ± 0.02	1.61 ± 0.06*
Blood viscosity (mPa·sec)		
For $\dot{\gamma}$ =		
75 sec^{-1}	4.3 ± 0.3	9.6 ± 0.5*
90 sec^{-1}	4.5 ± 0.3	8.6 ± 0.7*
150 sec^{-1}	3.8 ± 0.2	7.9 ± 0.6*
225 sec^{-1}	3.7 ± 0.2	7.0 ± 0.6*
Relative viscosity of blood		
For $\dot{\gamma}$ =		
75 sec^{-1}	3.1 ± 0.2	6.0 ± 0.3*
90 sec^{-1}	3.2 ± 0.2	5.3 ± 0.4*
150 sec^{-1}	2.7 ± 0.2	4.9 ± 0.4*
225 sec^{-1}	2.6 ± 0.2	4.4 ± 0.4*

All values are mean ± SEM.

** Significantly different compared to WKY group values for* $P < .05$.

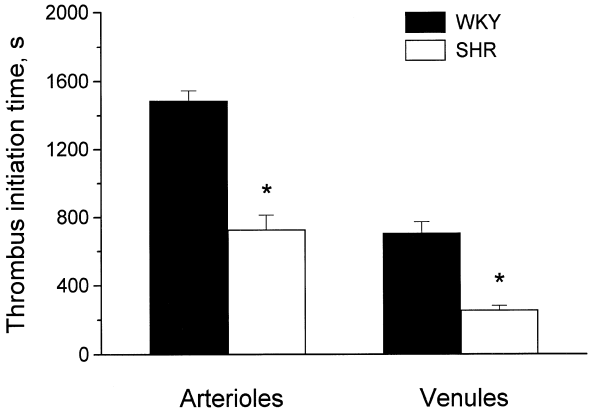

FIGURE 1. *The time for platelet thrombus initiation in arterioles and in venules of 12 week old WKY (n = 7) and SHR (n = 6) rats. *P < .05 for comparison between WKY and SHR groups. The ratio of the time to platelet thrombus appearances in the WKY group to that in the SHR is equal to 2.18 ± 0.19 at a wall shear of 2000 sec^{-1} (in arterioles), which is significantly different than the ratio of 2.92 ± 0.25 at a wall shear of 270 sec^{-1} (in venules).*

Photoactivation of the intravascular FITC-BSA caused platelet thrombus formation and eventual vessel occlusion in arterioles and venules of both WKY and SHR groups. The time period for appearances of platelets adhered to the vessel wall was shorter in both arterioles and in venules in the SHRs compared to the arterioles and venules of the WKY group (Figure 1). The ratio of the time for platelet thrombus appearances in the WKY group to the time period for platelet thrombus initiation in SHRs was less at the wall shear rate of 2000 sec^{-1} (in arterioles) than at the wall shear rate of 270 sec^{-1} (in venules) (Figure 1).

Platelet thrombus growth time following thrombus initiation was significantly longer in the arterioles and in venules of SHRs compared to the arterioles and venules of the WKY rats (Figure 2). There was no significant difference found in the ratio of platelet thrombus growth time in the WKY group to platelet thrombus growth time in SHRs at the wall shear rate of 2000 sec^{-1} (in arterioles) and at the wall shear rate of 270 sec^{-1} (in venules) (Figure 2).

The shear rate change in the SHR group was significantly increased in both the arterioles and venules compared to the arterioles and venules of WKY rats at respectively similar values of the wall shear rates (Figure 3). The ratio of shear rate change in the WKY group to shear rate change in the SHRs was higher at the wall shear rate of 2000 sec^{-1} (in arterioles) than at the wall shear rate of 270 sec^{-1} (in venules) (Figure 3).

The bleeding time following micropuncture of second-order venules was determined as an index of platelet aggregation following exposure to subendothelial collagen. Bleeding time was significantly shortened in all SHRs (44 ± 1.2 sec; n = 6) compared to the

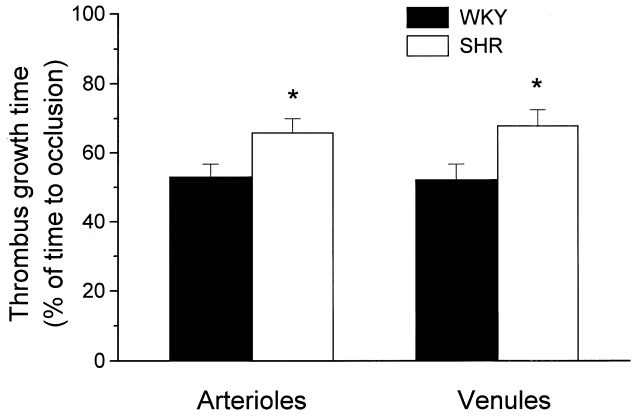

FIGURE 2. *The time of platelet thrombus growth following thrombus initiation in arterioles and venules of 12 week old WKY (n = 7) and SHR (n = 6) rats. *P < .05 for comparison between WKY and SHR groups. The ratio of platelet thrombus growth time in the WKY group to platelet thrombus growth time in the SHRs is equal to 0.83 ± 0.06 at a wall shear rate of 2000 sec^{-1} (in arterioles) and 0.79 ± 0.06 at a wall shear rate of 270 sec^{-1} (in venules). There is no significant difference between these ratios.*

value in the normotensive WKY (64.6 ± 3.1 sec; n = 7) animals.

DISCUSSION

In the current study, genetic hypertension (SHR) caused higher in vivo platelet responsiveness to thrombogenic stimuli in microvessels compared to the normotensive (WKY) animals. These results occurred in animals where mean arterial blood pressure and heart rate were significantly higher compared to WKY rats, but there was no difference in body weight (Table 1). Hematocrit and the number of circulating platelets in the spontaneously hypertensive rats were found to be higher than in the normotensive rats (Table 1). Blood and plasma viscosity (Table 2) and relative viscosity of blood (Table 2) were also increased in the SHR group as compared with the WKY group. Elevation of these systemic and blood rheological variables are typical for hypertensive disease.

The values of the shear rates used in the viscometry study were typical for rat cremaster muscle microvessels (venules). It is known that at the low shear rates relative blood viscosity could be used as an index of the degree of red blood cell aggregability.[20] An increased relative blood viscosity in the SHR group compared to that in the WKY group (Table 2) is in agreement with previous studies in patients with essential hypertension[1,4–6] and suggests that the development of hypertension increases the red blood cell aggregability in rats.

Platelet thrombus formation induced by photoactivation was studied at similar values of wall shear rates in arterioles (~2000 sec^{-1}) and venules (~270 sec^{-1}) to equalize the effect of shear forces. Similarities of shear rates in microvessels of SHR and WKY rats enabled us to minimize differences of the flow conditions in microvessels with similar internal diameters and investigate processes caused by chronic elevation of the mean arterial blood pressure (Table 1) in the SHR group compared to the WKY rat group.

The shorter time for platelet thrombus initiation in both arterioles and venules of SHRs compared to the WKY group (Figure 1) shows that platelet adhesion is enhanced during genetically induced hypertension in rats. Greater wall shear rates (~2000 sec^{-1}) in arterioles were associated with an increase in the time required for platelet adhesion to occur compared to the platelet adhesion at lower wall shear rates (~270 sec^{-1}) observed in venules (Figure 2). The significant difference in the ratio of the time period for platelet thrombus initiation in WKY rats to that in the SHRs in arterioles and in venules (Figure 1) suggests that the platelet adhesion is influenced by wall shear rate or functional differences between arteriolar and venular endothelium.

Although a portion of the time required for platelet thrombus growth following thrombus initiation was significantly prolonged in the microvessels of the SHR group compared to that in the WKY group (Figure 2), the ratios of platelet thrombus growth time in the WKY group to platelet thrombus growth time in SHRs at high (~2000 sec^{-1}) wall shear rates (arterioles) and at lower (~270 sec^{-1}) shear rates (venules) were not different (Figure 2). These results suggest that the alterations of the platelet aggregation are independent from wall shear rate changes. Even though in vitro platelet aggregation has been reported to be increased

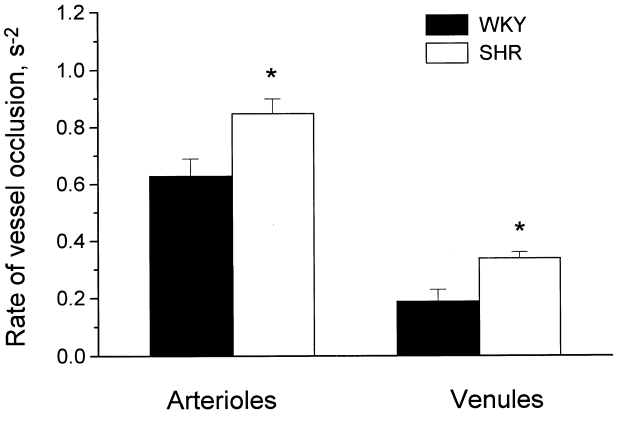

FIGURE 3. *The rate of occlusion of arterioles and venules induced by platelet thrombus in 12 week old WKY (n = 7) and SHR (n = 6) rats. *P < .05 for comparison between WKY and SHR groups. The ratio of shear rate change in the WKY group to the shear rate change in the SHRs is equal to 0.76 ± 0.05 at 2000 sec^{-1} (in arterioles), which is significantly different than the ratio of 0.57 ± 0.04 at a wall shear rate of 270 sec^{-1} (in venules).*

during hypertension,[7] the decreased in vivo platelet aggregation in the SHR group in the present study corresponds with other in vivo as well as in vitro work,[21] which points to a greater effect of the first stage of thrombogenesis, ie, platelet adhesion to vessel wall, during genetically induced hypertension.

The shear rate change which represents the overall thrombotic process, is increased in both arterioles and in venules of the SHRs compared to the WKY group (Figure 3). These results are in agreement with previous in vitro work, which shows that platelet thrombus formation is increased during the genetic hypertension in rats.[22] At lower wall shear rates (venules), platelet thrombus formation is increased more than at higher wall shear rates (arterioles) in the present study (Figure 1). The significant difference between arterioles and venules in the ratio of the shear rate change in the WKY group compared with the shear rate change in the SHRs (Figure 3) suggests that wall shear rate altered by genetic hypertension is a major factor in the increased platelet thrombus formation process seen.

Alteration of blood rheological properties is one of the mechanisms that results in increased thrombogenesis during hypertension. An increased concentration of adhesive protein vWF[23] and increased fibrinogen binding[7] in SHRs is another plausible mechanism for hypertension-induced enhanced thrombogenesis in the response to acute focal injury. Although vWF is thought to have a greater effect at higher wall shear rates (arterioles),[24,25] our previous results,[26] in agreement with Ross et al,[25] have shown vWF's importance at lower wall shear rates as well. Furthermore, evidence of decreased bleeding time seen during hypertension (see the Results section) suggest that increased platelet thrombus formation may be related to the changes in structural proteins of subendothelial collagen.[27,28]

The endothelial cells also have an active role in the modulation of thrombus formation. They release nitric oxide (NO), which is a vascular smooth muscle relaxant[29] and which inhibits platelet adhesion and aggregation in vitro.[31,31] Production of this antiaggregatory factor is decreased during the development of genetic hypertension in rats[12] and may be a mechanism that contributes to the increased thrombogenesis seen here (Figures 1, 3).

In summary, we have used two separate focal injury methods to study platelet-mediated hemostatic function during genetic hypertension. The results demonstrate increased thrombogenesis in the hypertensive rats that appears to be the result of increased platelet-to-endothelium adhesion and not platelet-to-platelet aggregation. These results coincide with slightly increased hematocrit (Table 1), increased red blood cell aggregability (Table 2), and a higher than normal numbers of platelets (Table 1). Although increased number of platelets is not a mechanism associated with increased thrombogenesis in the hypertensive phenotype,[32] it may be caused by changes in platelet function.[7] The present results demonstrate that the normal hemostatic function of platelets is changed by altered blood rheological and endothelial cell properties induced by chronically elevated mean arterial pressure. In conclusion, we suggest that the increased thrombus formation during the development of genetic hypertension is mainly the result of an altered platelet-to-endothelial cell interaction.

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REFERENCES

1. Letcher RL, Chien S, Pickering TG, et al: Elevated blood viscosity in patients with borderline essential hypertension. Hypertension 1983;5:757–762.
2. Zannad F, Stoltz J-F: Blood rheology in arterial hypertension. J Hyperten 1992;10(suppl 5):S69–S78.
3. Blann AD, Naqvi T, Waite M, et al: Von Willebrand factor and endothelial damage in essential hypertension. J Hum Hypertens 1993;7:107–111.
4. Letcher RL, Chien S, Pickering TG, et al: Direct relationship between blood pressure and blood viscosity in normal and hypertensive subjects; role of fibrinogen and concentration. Am J Med 1981;7:1195–1202.
5. Mchedlishvili G, Beritashvili N, Lominadze D, et al: Technique for direct and quantitative evaluation of erythrocyte aggregability in blood samples. Biorheology 1993;30:153–161.
6. Tsinamdzvrishvili B, Beritashvili N, Mchedlishvili G: Further insight into blood rheological disturbances in essential hypertension. Clin Hemorheol 1995;15(5):697–705.
7. Huzoor-Akbar' Kundu N, Kornhauser R: Normal thrombin binding leads to greater fibrinogen binding and increased platelet aggregation in spontaneously hypertensive rats. Life Sci 1993;53(26):1967–1974.
8. Nyrop M, Zweifler AJ: Platelet aggregation in hypertension and the effects of antihypertensive treatment. J Hypertens 1988;6:263–269.
9. Lip GJH, Beevers DG: Abnormalities of rheology and coagulation in hypertension. J Hum Hypertens 1994; 8(9):693–702.
10. Somova L, Mufunda J: Platelet activity and salt sensitivity in the pathogenesis of systemic (essential) hypertension in black afrikans. Clin Exp Hypertens 1993; 15(5):781–796.
11. Gleerup G, Vind J, Winther K: Platelet function and fibrinolytic activity during rest and exercise in borderline hypertensive patients. Eur J Clin Invest 1995;25(4): 266–270.
12. Li F, Joshua IG: Decreased arteriolar endothelium-derived relaxing factor production during the development of genetic hypertension. Clin Exper Hypertens 1993;15(3):511–526.

13. Chien S, Usami S, Dellenback RJ, Bryant CA: Comparative hemorheology: hematological implications of species differences in blood viscosity. Biorheology 1971;8: 35–57.

14. Sato M, Ohshima N: Platelet thrombus induced *in vivo* by filtered light and fluorescent dye in mesenteric microvessels of the rat. Thrombosis Res 1984;35:319–334.

15. Sato M, Ohshima N: Effect of wall shear rate on thrombogenesis in microvessels of the rat mesentery. Circ Res 1990;66:941–949.

16. Miller FN, Sims DE, Schuschke DA, et al: Differentiation of light-dye effects in the microcirculation. Microvasc Res 1992;44:166–184.

17. Wayland H, Johnson PC: Erythrocyte velocity measurement in microvessels by a two-slit photometric method. J Appl Physiol 1967;22:333–337.

18. Pittman RN, Ellsworth ML: Estimation of red cell flow in microvessels: consequences of the Baker-Wayland spatial averaging model. Microvasc Res 1986;32:371–388.

19. oude Egbrink MGA, Tangelder GJ, Slaaf DW, et al: Thromboembolic reaction following wall puncture in arterioles and venules of the rabbit mesentery. Thrombosis Haemostasis 1988;59:23–28.

20. Chien S, Usami S, Taylor HM, et al: Effects of hematocrit and plasma proteins on human blood rheology at low shear rates. J Appl Physiol 1966;21:81–87.

21. Takiguchi Y, Wada K, Nakashima M: Hemodynamic effects on thrombogenesis and platelet aggregation in spontaneously hypertensive rats. Clin Exp Hypertens 1993;15(1):197–208.

22. Cignarella A, Bertozzi D, Zaarour C, et al: Antithrombotic activity of nicardipine in spontaneously hypertensive rats. Pharmacol Res 1994;30(3):273–280.

23. McCarron RM, Doron DA, Siren AI, et al: Agonist-stimulated release of von Willebrand factor and procoagulant factor VIII in rats with and without risk factors for stroke. Brain Res 1994;647(2):265–272.

24. Ruggeri ZM: Mechanisms of shear-induced platelet adhesion and aggregation. Thrombosis Haemostasis 1993; 70(1):119–123.

25. Ross JM, McIntaire LV, Moake JL, et al: Platelet adhesion and aggregation on human type VI collagen surfaces under physiological flow conditions. Blood 1995; 85(7):1826–1835.

26. Lominadze DG, Saari JT, Miller FN, et al: *In vitro* platelet adhesion to endothelial cells at low shear rates during copper deficiency in rats. J Trace Elem Exp Med (in press).

27. Weber KT, Eghbali M: Collagen matrix synthesis and degradation in the development and regression of left ventricular hypertrophy. Cardiovasc Rev Rep 1991;12: 61–69.

28. Diez J, Laviades C, Monreal I, et al: Toward the biochemical assessment of myocardial fibrosis in hypertensive patients. Am J Cardiol 1995;76(13):14D–17D.

29. Koller A, Messina EJ, Wolin MS, et al: Endothelial impairment inhibits prostaglandin and EDRF-mediated arteriolar dilation *in vivo.* Am J Physiol 1989; 257(Heart Circ Physiol 26):H1966–H1970.

30. Radomski MW, Palmer RMJ, Moncada S: The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br J Pharmacol 1987;92:639–646.

31. Radomski MW, Palmer RMJ, Moncada S: Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 1987;ii:1057–1058.

32. Reed JP, Hendley ED: Blood cell changes in spontaneously hypertensive rats are not all associated with the hypertensive phenotype. J Hypertens 1994;12:391–399.