Simultaneously Increased TxA₂ Activity in Isolated Arterioles and Platelets of Rats With Hyperhomocysteinemia

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Abstract—We aimed to elucidate the effect of hyperhomocysteinemia (HHcy) on the synthesis of prostaglandins in rat skeletal muscle arterioles and platelets. Male Wistar rats were divided into 2 groups: (1) control rats, with plasma Hcy levels of 6.5±0.5 μmol/L (n=50) and (2) rats with HHcy, induced by daily intake of 1 g/kg body weight methionine in the drinking water for 4 weeks (plasma Hcy levels were 20.6±3.0 μmol/L, P<0.01 versus controls; n=50). Arterioles (diameter ≈130 μm) were isolated from the gracilis muscle, cannulated, and pressurized (at 80 mm Hg), and changes in their diameters were followed by video microscopy. Constrictions to bradykinin (BK; 10⁻⁹ to 10⁻⁷ mol/L) were significantly greater in HHcy than in control rat arterioles (at 10⁻⁹ mol/L BK, changes were 11±3% in control and 41±9% in HHcy rats). The cyclooxygenase inhibitor indomethacin (10⁻⁵ mol/L), the prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂) receptor antagonist SQ 29,548 (10⁻⁸ mol/L), or the TxA₂ synthase inhibitor furegrelate (5×10⁻⁶ mol/L) significantly decreased constrictions to BK in both groups but more so in HHcy arterioles, thus eliminating the difference between responses of HHcy and control arterioles. Constrictions to U46619 (a TxA₂ analogue) were significantly greater in HHcy than in control arterioles (at 10⁻⁷ mol/L U46619, values for controls were 33±2% and 54±3% for HHcy). Endothelium removal or indomethacin treatment attenuated constrictions to U46619 in HHcy arterioles and eliminated the difference in responses. Also, aggregation of platelets from HHcy rats to collagen and ADP was significantly enhanced compared with controls (with 5 μg/mL collagen: controls, 23±5%; HHcy, 49±5%; with 10⁻⁷ mol/L ADP: controls, 25±3%; HHcy, 35±3%). Indomethacin or SQ 29,548 caused greater inhibition of aggregation of HHcy platelets compared with controls, thereby eliminating the differences between the 2 groups. Thus, HHcy enhances TxA₂ synthesis both in the arteriolar endothelium and platelets. By promoting vascular constriction and platelet aggregation simultaneously, these alterations are likely to contribute to the atherothrombotic vascular diseases described in HHcy. (Arterioscler Thromb Vasc Biol. 2000;20:1203-1208.)

Key Words: homocysteine • methionine • endothelium • arterioles • platelet aggregation

Several epidemiological studies have shown an association between elevated plasma homocysteine (Hcy) concentrations and atherothrombotic vascular diseases.¹⁻⁴ In the general human population, mild hyperhomocysteinemia (HHcy) is rather common (1:70) and is found in ≈30% of individuals with coronary, cerebrovascular, or peripheral atherothrombotic disease.³,⁴ Hcy is a thiol-containing amino acid that is formed from the essential amino acid methionine. Plasma Hcy concentration may increase in different pathophysiological conditions, including deficiency of vitamins such as folic acid, cyanocobalamin, and pyridoxal phosphate; and in the presence of various enzyme abnormalities (cystathionine β-synthetase, temperature-sensitive methylenetetrahydrofolate reductase; for further information, see Reference 2), all of which participate in the metabolism of methionine and Hcy. The mechanism(s) by which elevated Hcy promotes atherothrombotic vascular diseases is still not clearly elucidated and is likely to be multifactorial.⁵,⁶ It has been shown that HHcy stimulates smooth muscle proliferation⁷,⁸ and promotes LDL oxidation.⁹ There are reports demonstrating that HHcy impairs the vasoactive function of the endothelium as well. In experimental animals, severe HHcy resulted in endothelial cell loss.¹⁰ The adverse effect of Hcy was also confirmed by in vitro studies on cultured endothelial cells.¹¹,¹² In conduit arteries of patients with HHcy, vasodilation associated with reactive hyperemia is impaired,¹³⁻¹⁶ whereas reduced acetylcholine (ACH)-induced dilation was demonstrated in carotid arteries isolated from HHcy monkeys.¹⁷,¹⁸

Less is known, however, of how the function of microvessels is altered in HHcy. We have recently demonstrated that elevated levels of Hcy in rats interfere with skeletal muscle arteriolar responses, which are mediated or modulated by endothelium-derived NO.¹⁹ Alterations of NO synthesis/release are frequently accompanied by enhanced constrictor responses due to changes in other endothelial pathways, such

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as an increased synthesis of prostaglandin \( \text{H}_2 \) thromboxane \( \text{A}_2 \) (PG\( \text{H}_2/\text{TxA}_2 \)).\(^{20} \) Interestingly, previous studies found an enhanced platelet activation to infusion of collagen, which caused a marked reduction of hindlimb blood flow in HHcy compared with control monkeys.\(^{17} \) The underlying mechanism for this alteration could be due to increased TxA\(_2\) synthesis of platelets in HHcy.\(^{21} \) Thus, it is likely that in HHcy, there are changes in vascular arachidonic acid metabolism as well, yet the effect of HHcy on endothelial prostaglandin synthesis in arterioles has not been elucidated.

On the basis of these findings, we hypothesized that in HHcy, the synthesis of PG\( \text{H}_2 \) and/or TxA\(_2\) is simultaneously upregulated in vascular tissue and platelets. To test this hypothesis, we measured the arteriolar responses of control and HHcy rats to bradykinin (BK), an agent that is known to initiate the synthesis of arachidonic acid metabolites, such as PG\( \text{H}_2 \) and TxA\(_2\), in the endothelium.\(^{22–24} \) BK has pathological implications as well, since it is an important mediator of various inflammatory processes that are also associated with atherosclerotic vascular diseases.\(^{25} \) Also, we investigated the arteriolar responses to intraluminal pressure and U46619, a stable TxA\(_2\) analogue, and the aggregation of platelets from the same animals in response to ADP and collagen, which are known to depend, at least in part, on TxA\(_2\) synthesis.

Methods

In male Wistar rats weighing \( \approx 150 \) g (purchased from Charles River Co), moderate HHcy was induced by administration of L-methionine (1 g · kg body weight \(^{-1} \) · d\(^{-1} \)) and succinylsulfathiazole (0.1 g · kg body weight \(^{-1} \) · d\(^{-1} \)) in the drinking water for a period of 4 weeks (\( n = 50 \)), as described previously.\(^{17–19} \) Succinylsulfathiazole was used to limit the release of Hcy from blood cells. Serum was then stored at \(-20^\circ \text{C} \) until assayed. Total Hcy concentrations were measured by high-performance liquid chromatography technique with fluorometric detection.\(^{19,26} \) In brief, 240 \( \mu \)L of serum and 60 \( \mu \)L of internal standard (\( \text{N-acetyl-L-cysteine, 50 } \mu\text{mol/L final concentration} \)) were reduced for 30 minutes at \( 4^\circ \text{C} \) with 30 \( \mu \)L of \( 1 \) mol/L borate buffer (pH 9.5), and 50 \( \mu \)L of 1 mg/mL 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate. After derivatization at 60°C (1 hour), the sample was analyzed on a high-performance liquid chromatograph (JASCO International Co Ltd) equipped with a fluorescence detector (LC 1255, GBC Scientific Equipment Pty Ltd). Separation was carried out on a 200\( \times \)4.6-mm \( \times 5 \)-\( \mu \)m Nucleosil C18 column. The eluent was 0.1 mol/L acetate buffer (pH 4.0) containing 2% methanol. The fluorescence intensities were measured with excitation at 386 nm and emission at 516 nm.

Analysis of Plasma Fatty Acid Content, Lipid Peroxidation Products, and Lipoprotein Susceptibility to Oxidation

Plasma lipids were extracted\(^{27} \) and the extracts were transmethylated with BF\(_3\)/methanol. The fatty acid methyl esters were separated by capillary gas chromatography (Chrompack CP 9000 column; wall-coated open tubular fused-silica 50-m \( \times \)0.25-mm CP-SIL88). Plasma lipid peroxidation was measured as thio-agaric acid-reactive substances and expressed in malondialdehyde equivalents.\(^{27} \) Briefly, each plasma sample (200 \( \mu \)mol/L) was mixed with thiobarbituric acid reagent (1 mL), heated (100°C, 20 minutes), and centrifuged, and the absorbance of the supernatant fraction was measured at 532 nm. LDL and VLDL were isolated by ultracentrifugation and were tested for their susceptibility to in vitro copper-induced oxidation.\(^{27} \) In brief, after isolation, lipoproteins were dialyzed for 20 hours at 4°C in the dark, against PBS (pH 7.4, purged with \( \text{N}_2 \)). The kinetics of LDL oxidation to \( \text{CuCl}_2 \) (15 \( \mu \)mol/L) was determined by monitoring the change in diene absorption at 234 nm on a UV spectrophotometer.

Isolation of Arterioles

Experiments were conducted on isolated arterioles (\( \approx 130 \mu \)m active and \( \approx 180 \mu \)m passive diameters at 80 mm Hg) of rat gracilis muscle as described previously.\(^{19,20,28} \) In brief, on the fourth week, the rats were fasted overnight and then anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg). The gracilis muscle was exposed and isolated from surrounding tissues. The muscle then was dissected out; placed in a silicone-lined Petri dish containing cold (0°C to 4°C) physiological saline composed of (in mmol/L) 110 \( \text{NaCl}, 5.0 \text{KCl}, 2.5 \text{CaCl}_2, 1.0 \text{MgSO}_4, 1.0 \text{KH}_2 \text{PO}_4, 10.0 \text{dextrose}, \) and 24.0 \( \text{NaHCO}_3; \) and equilibrated with a gas mixture of 10% \( \text{O}_2 \) and 5% \( \text{CO}_2 \), balanced with \( \text{N}_2 \), at pH 7.4. With the use of microsurgery instruments and an operating microscope, a 1.5-mm-long segment of the first-order arteriole running intramuscularly was isolated and transferred into an organ chamber containing 2 glass micropipettes filled with physiological saline solution. From a reservoir, the vessel chamber (15 mL) was continuously supplied with physiological saline solution at a rate of 20 mL/min. After the vessel had been mounted on the proximal micropipette and was secured with sutures, the perfusion pressure was raised to 20 mm Hg to clear the red blood cells from the lumen. Then the other end of the vessel was mounted on the distal pipette. Both micropipettes were connected with silicone tubing to an adjustable physiological saline solution reservoir. Pressures on both sides were measured by electromanometers. The perfusion pressure was slowly (over \( \approx 1 \) minute) increased to 80 mm Hg. The temperature was set at 37°C by a temperature controller (Grant Instruments), and the vessel was allowed to equilibrate for \( \approx 1 \) hour.

Experimental Protocols

After the equilibration period, changes in the diameter of arterioles in response to increases in perfusion pressure (from 10 to 20 and from 20 to 140 mm Hg in 20-mm Hg steps) were measured under zero-flow conditions.\(^{29} \) The pressure was maintained for 5 minutes at each pressure step to allow the vessel to reach a steady-state diameter. At the conclusion of each experiment, the suffusion solution was changed to a \( \text{Ca}^{2+} \)-free physiological saline solution, which contained sodium nitroprusside (SNP, \( 10^{-7} \) mol/L) and EGTA (1.0 \( \mu \)mol/L); the vessel was incubated for 10 minutes and the pressure steps were repeated to obtain the maximum passive diameter at each pressure value (pressure–passive diameter relationship). The diameter was measured with a micrometer and recorded on a chart recorder (Radelkis).

In preliminary studies, we selected doses of BK that elicited constrictions of gracilis arterioles. Responses of arterioles of control and HHcy rats to BK (10\(^{-5} \) to \( 10^{-7} \) mol/L) were compared in the absence and presence of indomethacin (10\(^{-6} \) mol/L), an inhibitor of prostaglandin synthesis; the specific TxA\(_2\) receptor antagonist SQ 29,548 (10\(^{-7} \) mol/L); or the specific TxA\(_2\) synthase inhibitor furegrelate\(^{30} \) (Un63557A, 5 \( \times 10^{-8} \) mol/L).

In separate experiments, the constrictor responses of arterioles of control and HHcy rats to the stable TxA\(_2\) analogue U46619 (10\(^{-11} \) to \( 5 \times 10^{-8} \) mol/L) were compared before and after endothelium removal. The endothelium of the arteriole was removed by perfusion of the vessel with air for \( \approx 1 \) minute at a perfusion pressure of 20 mm Hg. The arteriole was then perfused with physiological saline solution to clear the debris. The perfusion pressure was then raised to 80 mm Hg for 30 minutes to establish a stable tone. The efficacy of endothelial denudation was ascertained by arteriolar responses to ACh (10\(^{-7} \) mol/L, an endothelium-dependent agent) and SNP (10\(^{-3} \) mol/L, an endothelium-independent agent) before and after admin-
istration of the air bolus. The infusion of air resulted in loss of function of the endothelium, as indicated by the absence of dilation to ACh, whereas dilation to SNP remained intact.

In further experiments, the constrictor responses of arterioles of control and HHcy rats to U46619 were compared in the presence and absence of SQ 29,548 (10⁻⁶, 2×10⁻⁷, 10⁻⁸, and 10⁻⁹ mol/L). The U46619 (10⁻⁶ to 10⁻⁸ mol/L) and SQ 29,548 (10⁻⁶ and 2×10⁻⁸ mol/L) interaction being apparently competitive, the negative logarithm (pA₂) of the equilibrium dissociation constant (Kᵣ) was calculated for the antagonist. The Kᵣ was calculated from the equation Kᵣ=α(DR⁻¹), where α is the molar concentration of antagonist, and DR, the dose ratio, is the measure of the rightward shift of the agonist dose-response curve. In separate experiments, the constrictor responses of arterioles of control and HHcy rats to U46619 (10⁻¹¹ to 5×10⁻⁸ mol/L) were compared in the absence and presence of indomethacin (10⁻³ mol/L) or furegrelate (5×10⁻⁶ mol/L).

All drugs were added to the vessel chamber, and final concentrations are reported. After responses to each drug subsided, the system was flushed with physiological saline solution. Changes in diameter were expressed as a percentage of baseline.

Platelet Aggregation Studies
Aggregation of platelets was studied as described previously. In brief, heparinized blood was removed from the aorta of the same rats that were used to study isolated arterioles. Platelet-rich plasma (PRP) was prepared from the remaining volume of blood by centrifugation at 1600g for 10 minutes. The concentration of platelets in PRP was determined by light microscopy in a counting chamber and was adjusted to 0.3×10⁹/mL by diluting it with PPP. Both PRP and PPP were kept at 22°C and used within 1.5 hours of blood collection. Cuvettes containing 0.5 mL of PRP were placed in a turbidimetric aggregometer (Mikron M304) under stirring (1000 rpm at 37°C). Aggregation was started by addition of collagen (2.5 to 20 μg/mL) or ADP (10⁻⁵ to 3×10⁻⁶ mol/L). Where indicated, indomethacin (5×10⁻⁵ mol/L) or SQ 29,548 (10⁻⁶ mol/L) was given 6 minutes before the start of platelet activation. The extent of aggregation was expressed as a percentage of the maximal change in optical density represented by autologous PPP.

U46619 was obtained from Cayman Chemical Co, and SQ 29,548 was obtained from Bristol-Myers Squibb; all other salts and chemicals were obtained from Sigma-Aldrich Co. Solutions were prepared on the day of the experiment. Data are expressed as mean±SEM. Statistical analyses were performed by 2-way ANOVA for repeated measures followed by Tukey’s post hoc test or Student’s t test, as appropriate. *P<0.05 was considered statistically significant.

Results
The methionine-rich diet elicited a significantly greater concentration of Hcy in the plasma of HHcy compared with control rats (20.6±2.30 and 6.5±0.5 μmol/L, respectively; P<0.01). There was no difference in the body weight between control rats and methionine-fed rats (382±10 and 365±15 g, respectively; NS).

Plasma Fatty Acid Composition and Lipid Peroxidation Products
The 20:4(n-6) arachidonic acid content was significantly increased in plasma lipids of HHcy (n=6) compared with control rats (24.6±2.35% and 16.3±1.7%, respectively; P<0.05). The lipoprotein oxidation rate was 17.0±24.0 and 17.5±8.0 nmol · min⁻¹ · mg LDL protein⁻¹; NS), and the thiobarbituric acid–reactive substances content was 12.5±1.5 and 11.8±2.0 nmol/mL (NS) in control and HHcy rats, respectively.

Figure 1. Diameters of isolated skeletal muscle arterioles from control (C, n=10) and HHcy (n=10) rats as a function of perfusion pressure in the presence (circles) and absence (passive; triangles) of Ca²⁺ in the superfusion solution. Data are mean±SEM.

Pressure-Diameter Relationships of Arterioles
Isolated arterioles of gracilis muscle from both control and HHcy rats developed active tone in response to step increases in intraluminal pressure (10 to 140 mm Hg) without the use of any vasoactive agent (Figure 1). Initially, the diameter of these vessels increased from ≈100 to ≈150 μm in response to an increase in intravascular pressure from 10 to 40 mm Hg. Beyond this point, further increases in pressure resulted in constrictions of arterioles. There was no significant difference in pressure-induced responses of arterioles from the 2 groups of rats. In the absence of Ca²⁺ and in the presence of 10⁻⁴ mol/L SNP, the pressure–passive diameter relationship in each arteriole was also obtained (Figure 1). In this condition, step increases in pressure elicited increases in diameter of arterioles, reaching a plateau at 100 mm Hg pressure.

Arteriolar Responses to BK and U46619
In a dose-dependent manner, BK (10⁻⁹ to 10⁻⁷ mol/L) elicited significantly greater constrictions in arterioles from HHcy than those from control rats (Figure 2A). To elucidate the

Figure 2. Constrictions to BK in arterioles of control (C) and HHcy rats under control conditions (A) or in the presence of the cyclooxygenase inhibitor indomethacin (10⁻⁵ mol/L; B), the TxA₂ receptor antagonist SQ 29,548 (10⁻⁶ mol/L; C), or the TxA₂ synthase inhibitor furegrelate (5×10⁻⁶ mol/L; D). Data are mean±SEM (n=5 to 10). *Significant (P<0.05) difference from control values.
nature of mediators released in response to BK, we tested arteriolar responses in the presence of the prostaglandin synthesis inhibitor indomethacin and the specific PGH2/TxA2 receptor inhibitor SQ 29,548. Incubation with indomethacin or SQ 29,548 elicited significantly greater inhibition of BK-induced constrictions in arterioles of HHcy than of control rats and eliminated the difference between the responses in the 2 groups (Figures 2B and 2C). To further identify the constrictor prostaglandin released in response to BK, we utilized the specific TxA2 synthase inhibitor furegrelate. Furegrelate also elicited significantly greater inhibition of BK-induced constrictions in arterioles of HHcy than of control rats and eliminated the difference between the responses in the 2 groups (Figure 2D).

In a dose-dependent manner, the stable TxA2 analogue U46619 (10^{-9} to 10^{-6} mol/L) elicited significantly greater constrictions of arterioles from HHcy than those from control rats (Figure 3A). Increasing doses of SQ 29,548 (10^{-8} to 10^{-7} mol/L) elicited gradual decreases in arteriolar constrictions to U46619, whereas 10^{-6} mol/L abolished the responses. A selected dose of SQ 29,548 (10^{-8} mol/L) caused a parallel rightward shift of U46619 dose-response curves in both control and HHcy arterioles (Figure 3B). From the parallel shift and assuming a competitive interaction, a pA2 of 8.69±0.18 and 8.56±0.17 (in control and HHcy arterioles, respectively; NS) was calculated.

Indomethacin significantly decreased constrictions to U46619 in arterioles of HHcy rats. In the presence of indomethacin, constrictions to U46619 were not significantly different between control and HHcy arterioles (Figure 3C). To elucidate the cellular source of enhanced release of TxA2, the endothelium of arterioles was removed. Endothelium removal significantly attenuated constrictions to U46619 in arterioles of HHcy rats. In the absence of the endothelium, there was no significant difference between responses to U46619 in control and HHcy rats (Figure 3D).

Platelet Aggregation Studies

Next we investigated the effect of collagen on aggregation of platelets isolated from the blood of HHcy and control rats. We found that collagen-induced aggregation of platelets from HHcy rats was significantly enhanced compared with that of controls (at 5 μg/mL, controls =23±5%; HHcy =49±5%; Figure 4A). Similarly, we found that ADP elicited significantly greater aggregation in HHcy than in control platelets (at 10^{-7} mol/L, controls =25±3%; HHcy =35±3%; Figure 4B). Indomethacin or SQ 29,548 caused greater inhibition of collagen- and ADP-induced aggregation of HHcy platelets compared with controls (Figure 5).

Discussion

The salient findings of this study are that elevated plasma Hcy concentration in rats resulted in enhanced arteriolar
constrictions to BK and the stable TxA$_2$ analogue U46619 and an augmented collagen- and ADP-induced aggregation of platelets. The underlying mechanism for these alterations is likely an increased TxA$_2$ synthesis in the arteriolar endothelium and platelets in HHcy.

Epidemiological studies have revealed that an elevated level of Hcy is an independent risk factor of human atherosclerosis. Previous studies suggested an impaired endothelium-associated dilation in conduit arteries of patients with HHcy. In addition, the endothelium-dependent relaxation of carotid artery and an increase in hindlimb circulation to ACh were shown to be reduced in monkeys with diet-induced HHcy. The endothelium is important in the local control of blood flow, and several cardiovascular diseases, such as hypertension and atherosclerosis, has been documented. Recently, we have demonstrated that NO-mediated endothelial responses are impaired in skeletal muscle arterioles of HHcy rats, suggesting an important alteration in endothelial regulation of arteriolar tone.

Interestingly, impairment of endothelial NO synthesis is often associated with alterations of vascular synthesis of prostaglandins. For example, in hypertension, decreased NO synthesis and increased PGH$_2$/TxA$_2$ production are present simultaneously in skeletal muscle arterioles. Thus, we hypothesized that the impaired endothelial NO bioavailability in skeletal muscle arterioles would also lead to enhanced PGH$_2$/TxA$_2$ synthesis in HHcy. The methionine diet utilized in the present study increased plasma Hcy levels by 3-fold, reaching a concentration similar to what was shown to be associated with an increased risk of vascular disease in humans.

In the present experiments, we found no significant differences between the pressure-diameter curves of the 2 groups of arterioles (Figure 1), suggesting that the myogenic tone and the contractile activity of arteriolar smooth muscle are not affected in general by this model of HHcy, and that the observed alterations in arteriolar responses are not due to changes in the function of smooth muscle.

To test the role of PGH$_2$/TxA$_2$ in the impaired responses of arterioles, we investigated responses to BK, which are known to be mediated by multiple pathways, including eicosanoids. Previous studies in isolated porcine iliac arteries and renal afferent arterioles demonstrated that 10$^{-9}$ to 10$^{-7}$ mol/L BK elicited constriction, primarily due to the release of cyclooxygenase products from the endothelium, whereas higher concentrations (>10$^{-5}$ mol/L) of BK exerted a direct effect on the vascular smooth muscle. We confirmed these findings by demonstrating that in rat gracilis muscle arterioles, BK-induced constrictions were mediated primarily by constrictor prostanoids, since both inhibition of prostaglandin synthesis with indomethacin and blocking the PGH$_2$/TxA$_2$ receptors with SQ29,548 (Figures 2B and 2C) inhibited these responses. We found that HHcy significantly enhanced BK-induced constrictions of arterioles (Figure 2A), which were likely due to an increased synthesis of PGH/TxA$_2$, as both indomethacin and SQ 29,548 substantially inhibited the responses, thereby eliminating the difference between HHcy and control arterioles. Furthermore, the findings that the specific TxA$_2$ synthase inhibitor furegrelate inhibited BK-induced constrictions and also eliminated the difference between responses in control and HHcy arterioles (Figure 2D) indicate that TxA$_2$ is the primary constrictor prostaglandin synthesized to BK in arterioles of HHcy rats.

We also found that constrictions to the stable TxA$_2$ analogue U46619 were enhanced in HHcy (Figure 3A). Because SQ 29,548 caused a similar rightward shift in the dose-response curves of control and HHcy arterioles to U46619 (as reflected by the calculated pA$_2$ values; Figure 3B), we assumed that an altered TxA$_2$ receptor sensitivity was unlikely to have contributed to the increased constriction to BK and U46619 in HHcy. Previously, it had been shown that vascular responses to U46619 were modulated by a further release of endothelial factors, such as TxA$_2$. Thus, the increased constriction to U46619, as well as to BK in HHcy, is likely due to an additional release of TxA$_2$ from the arteriolar endothelium. Indeed, the findings that indomethacin or removal of the endothelium decreased constrictions to U46619 in HHcy but not in control arterioles support this hypothesis (Figures 3C and 3D). Thus, arteriolar release of TxA$_2$ may contribute to the increased urinary excretion of TxB$_2$, the metabolite of TxA$_2$, that has been demonstrated in patients with genetic HHcy. In the present study, we aimed to elucidate whether alterations in platelet function were also related to increased TxA$_2$ activity in HHcy, independent of vascular mechanisms.

To that purpose, platelets were isolated from the blood of control and HHcy rats used for vascular studies, and agonist-induced platelet aggregation in the 2 groups was compared. Collagen and ADP are known to elicit platelet aggregation by a mechanism that involves TxA$_2$ synthesis/release. We found that collagen- and ADP-induced platelet aggregation was significantly enhanced in HHcy rats (Figure 5) and that either inhibition of cyclooxygenase or blocking of the TxA$_2$ receptors (Figure 5) eliminated the difference between aggregation of platelets from HHcy and control rats. These results indicate that the increased platelet aggregation in HHcy is most likely due to an enhanced formation of TxA$_2$ in platelets. Earlier studies also demonstrated an increased ADP-induced aggregation of platelets associated with an enhanced TxA$_2$ biosynthesis in rats with HHcy. Collectively, these studies suggest that simultaneous elevation of TxA$_2$ synthesis in endothelial cells and platelets is responsible for the increased urinary excretion of TxB$_2$ demonstrated in human HHcy.

One of the mechanisms that might favor the formation of TxA$_2$ is an increased formation of arachidonic acid. Indeed, we confirmed earlier findings by showing an increased level of arachidonic acid in the plasma of HHcy rats. This could be due to an elevated level of reactive oxygen species shown to be present in HHcy. Homocysteine, by the autooxidation of the sulfhydryl group, may promote the generation of oxygen free radicals, resulting in oxidative stress. In addition, homocysteine may decrease the intracellular level of glutathione and glutathione peroxidase, which are responsible for the elimination of oxygen free radicals. An enhanced level of reactive oxygen species is known to interfere with NO, a mechanism that may be responsible for the impaired arteriolar responses to ACh and histamine in HHcy. Also, NO and superoxide can form peroxynitrite, which may interfere with PGH$_2$ synthase promoting the elevation of TxA$_2$, especially when the level of its precursor arachidonic acid is elevated.

In conclusion, in HHcy, the reduced endothelial release of NO together with the simultaneously increased TxA$_2$ synthesis in arterioles and platelets could interfere with endothelial regulation of blood flow and enhance platelet aggregation, thereby predisposing the circulatory system to atherothrombotic alterations.
References


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