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: 10⁻¹¹ 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 (10⁻³ mol/L), the prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂) receptor antagonist SQ 29,548 (10⁻⁶ 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 10⁻⁶ 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 as...
as an increased synthesis of prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂).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 TxA₂ 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 PGH₂ and/or TxA₂ 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 PGH₁ and TxA₂, 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 TxA₂ 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 TxA₂ synthesis.

Methods

In male Wistar rats weighing ≈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 avoid bacterial proliferation and subsequent folate neoproduction. The doses administered were based on average daily fluid intake. Control animals (n=50) had free access to water. Animals were housed separately, fed standard rat chow, and were weighed at the start and end of the 4-week period.

Determination of Serum Hcy

Blood was collected from the aortas of fasted rats. It was immediately cooled on ice and centrifuged at 3000g for 20 minutes at 4°C to limit the release of Hcy from blood cells. Serum was then stored at −20°C until assayed. Total Hcy concentrations were measured by a high-performance liquid chromatography technique with fluorometric detection.¹⁹⁻²⁶ In brief, 240 µL of serum and 60 µL of internal standard (N-acetyl-L-cysteine, 50 µmol/L final concentration) were reduced for 30 minutes at 4°C with 30 µL of tr-i-n-butylphosphine (10%). Deproteinization was performed with 300 µL of 10% trichloroacetic acid. After centrifugation, 100 µL of clear supernatant was mixed with 20 µL of 1.55 mol/L NaOH, 250 µL of 0.125 mol/L borate buffer (pH 9.5), and 50 µL 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 200×4.6-mm×5-µm 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 extracted²⁷ and the extracts were transmethylated by capillary gas chromatography (Chrompack CP 9000 column: wall-coated open tubular fused-silica 50-m×0.25-mm CP-SIL88). Plasma lipid peroxidation was measured as thiobarbituric acid–reactive substances and expressed in malondialdehyde equivalents.²⁷ In brief, each plasma sample (200 µmol/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 N₂). The kinetics of LDL oxidation to CuCl₂ (15 µmol/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 (~130 µm active and ~180 µm 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 NaCl, 5.0 KCl, 2.5 CaCl₂, 1.0 MgSO₄, 1.0 KH₂PO₄, 10.0 dextrose, and 24.0 NaHCO₃; and equilibrated with a gas mixture of 10% O₂ and 5% CO₂, balanced with N₂, 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 ~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 ~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.²⁸ 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 perfusion solution was changed to a Ca²⁺-free physiological saline solution, which contained sodium nitroprusside (SNP, 10⁻⁶ mol/L) and EGTA (1.0 mmol/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 microangiometer 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 (10⁻⁴ to 10⁻⁷ mol/L) were compared in the absence and presence of indomethacin (10⁻⁴ mol/L), an inhibitor of prostaglandin synthesis; the specific TxA₂ receptor antagonist SQ 29,548 (10⁻⁶ mol/L); or the specific TxA₂ synthase inhibitor furegrelatin (163557A, 5×10⁻⁶ mol/L).

In separate experiments, the constrictor responses of arterioles of control and HHcy rats to the stable TxA₂ analogue U46619 (10⁻¹¹ to 5×10⁻⁶ mol/L) were compared before and after endothelium removal. The endothelium of the arteriole was removed by perfusion of the vessel with air for ~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 (10⁻⁵ mol/L, an endothelium-independent agent) before and after admin-
istration of the air bolus.29 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 (10–3 mol/L) and SQ 29,548 (10–3 mol/L 2) and 2×10–6 mol/L SQ 29,548 (10–3 and 2×10–6 mol/L) interaction being apparently competitive, the negative logarithm (pA2) of the equilibrium dissociation constant (Kd) was calculated for the antagonist.31,32 The Kd was calculated from the equation Kd = Δ(DR – 1), where a 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.21,33,34 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 obtained by centrifugation of blood at 250 g 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 (10–3 to 3×10–6 mol/L). Where indicated, indomethacin (5×10–3 mol/L) or SQ 29,548 (10–3 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 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

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 (n=6) rats (24.6±2.5%; and 16.3±1.7%, respectively; P<0.05). The lipoprotein oxidation rate was 17.2±0.4 and 17.5±0.8 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.

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.

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 (10–3 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 arterioles from 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% and 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% and 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 TxA2 analogue U46619 and an augmented collagen- and ADP-induced aggregation of platelets. The underlying mechanism for these alterations is likely an increased TxA2 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.1-4 Previous studies suggested an impaired endothelium-associated dilation in conduit arteries of patients with HHcy.13-16 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.18 The endothelium is important in the local control of blood flow,20,29,35 and in several cardiovascular diseases, such as hypertension30 and atherosclerosis,29 an impaired endothelial regulation of microvascular tone 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.36,37 For example, in hypertension, decreased NO synthesis and increased PGH2/TxA2 production are present simultaneously in skeletal muscle arterioles.20,38 Thus, we hypothesized that the impaired endothelial NO bioavailability in skeletal muscle arterioles would also lead to enhanced PGH2/TxA2 synthesis in HHcy. The methionine diet utilized in the present study19,21,34,39 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.1-4

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 PGH2/TxA2 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 arteries23 and renal afferent arterioles34 demonstrated that 10-9 to 10-7 mol/L BK elicited constriction, primarily due to the release of cyclooxygenase products from the endothelium.23,24 whereas higher concentrations (>10-7 mol/L) of BK exerted a direct effect on the vascular smooth muscle.40 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 PGH2/TxA2 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 PGH2/TxA2, as both indomethacin and SQ 29,548 substantially inhibited the responses, thereby eliminating the difference between HHcy and control arterioles (Figures 2B and 2C). Furthermore, the findings that the specific TxA2 synthase inhibitor furegrelate30 inhibited BK-induced constrictions and also eliminated the difference between responses in control and HHcy arterioles (Figure 2D) indicate that TxA2 is the primary constrictor prostaglandin synthesized to BK in arterioles of HHcy rats.

We also found that constrictions to the stable TxA2 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 pA2 values; Figure 3B), we assumed that an altered TxA2 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 TxA2.41,42 Thus, the increased constriction to U46619, as well as to BK in HHcy, is likely due to an additional release of TxA2 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 TxA2 may contribute to the increased urinary excretion of TxB2, the metabolite of TxA2, that has been demonstrated in patients with genetic HHcy.43-45 In the present study, we aimed to elucidate whether alterations in platelet function were also related to increased TxA2 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 TxA2 synthesis/release.33,46 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 TxA2 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 TxA2 in platelets. Earlier studies21 also demonstrated an increased ADP-induced aggregation of platelets associated with an enhanced TxA2 biosynthesis in rats with HHcy. Collectively, these studies suggest that simultaneous elevation of TxA2 synthesis in endothelial cells and platelets is responsible for the increased urinary excretion of TxB2 demonstrated in human HHcy.43-45

One of the mechanisms that might favor the formation of TxA2 is an increased formation of arachidonic acid. Indeed, we confirmed earlier findings21 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.21 Homocysteine, by the autoxidation of the sulfhydryl group, may promote the generation of oxygen free radicals, resulting in oxidative stress.12 In addition, homocysteine may decrease the intracellular level of glutathione and glutathione peroxidase, which are responsible for the elimination of oxygen free radicals.47,48 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.19 Also, NO and superoxide can form peroxynitrite, which may interfere with PGH2 synthase promoting the elevation of TxA2,49 especially when the level of its precursor arachidonic acid is elevated.

In conclusion, in HHcy, the reduced endothelial release of NO19 together with the simultaneously increased TxA2 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.
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