Altered Vascular Responses to Platelets From Hypercholesterolemic Humans

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Activated platelets release potent vasoactive factors. Previous studies have focused on mechanisms by which vascular abnormalities lead to altered responses of atherosclerotic arteries. We tested the hypothesis that the activation of platelets from hypercholesterolemic humans produces abnormal vascular responses. Responses to intraluminal and abluminal activation of platelets from normal subjects and type II hypercholesterolemic patients (total cholesterol, 274±16 [mean±SEM] mg/dl) were examined in carotid arteries from normal rabbits perfused in vitro. Intraluminal activation of normal platelets produced pronounced dilatation of arteries preconstricted with phenylephrine. Vasodilator responses to intraluminal activation of platelets from hypercholesterolemic patients were greatly impaired. Vasodilator responses to platelets from hypercholesterolemic patients were not restored to normal by LYS3,857 (10⁻⁵ M), a 5-hydroxytryptamine₁ serotonin antagonist, by SQ29,548 (10⁻⁴ M), a thromboxane A₂/prostaglandin H₂ receptor antagonist, or by apyrase (1.5 units/ml), an enzyme with ADPase activity. Abluminal activation of normal platelets produced modest constriction in quiescent arteries, and abluminal activation of platelets from hypercholesterolemic patients produced augmented vasoconstrictor responses. The major finding is that vasodilator responses to platelets from hypercholesterolemic patients are profoundly impaired, and vasoconstrictor responses to platelets from hypercholesterolemic patients are augmented. Mechanisms in addition to increased release of serotonin, thromboxane, and ADP appear to contribute to impaired vasodilator responses to hypercholesterolemic platelets. Thus, alteration of platelets by hypercholesterolemia, as well as altered vascular reactivity, may contribute to abnormal vascular responses in atherosclerosis. (Circulation Research 1993;72:737-743)

Key Words: hypercholesterolemia • platelets • carotid artery • ADP • serotonin • thromboxane • atherosclerosis

Platelets may adhere to atherosclerotic plaques, release vasoactive products, and produce vasoconstriction or perhaps vasospasm. Previous work has focused on the vessel wall and indicates that endothelial dysfunction in atherosclerosis plays a key role in altered vascular responses. We and others have suggested that augmented constrictor responses of atherosclerotic arteries to serotonin and thromboxane, combined with impaired dilator responses to ADP, may contribute to vasoconstrictor responses to platelets. We have observed recently that vascular responses to the activation of platelets in vivo are altered in atherosclerotic primates, with impairment of dilator responses and augmentation of constrictor responses.

Several studies have demonstrated that platelets from hypercholesterolemic humans and animals are hyperreactive and release increased amounts of serotonin, thromboxane, and ADP. It is not clear whether alteration of platelet function by hypercholesterolemia, as well as abnormalities of the vessel wall, contributes to abnormal vascular responses.

In this study, we tested the hypothesis that activation of platelets from hypercholesterolemic patients produces abnormal vascular responses. We also determined whether increased release of serotonin, thromboxane, and ADP accounts for altered vascular responses to activation of platelets from hypercholesterolemic patients.

Materials and Methods

Patients and Normal Subjects

Platelets were obtained from 14 nonsmoking patients with type II hypercholesterolemia (eight men and six women; age range, 21-54 years; mean, 38 years) with no overt clinical signs of atherosclerotic vascular disease. Patients were identified in the Lipid Clinic at the University Hospital, and classification as type II hypercholesterolemia was based on determination of fasting values of total cholesterol, triglyceride, and low density lipoprotein cholesterol. For comparison, platelets were obtained from 16 age- and gender-matched healthy, nonsmoking, normocholesterolemic volunteers.

Donors abstained from medications that are known to affect platelet function for at least 2 weeks before the studies. All donors had normal platelet counts, and none were taking medications that are known to affect lipid levels. Subjects with diabetes mellitus, hypertension, renal failure, liver disorder, and history of platelet
dysfunction were excluded. Informed consent was obtained from all subjects.

**Platelet Preparation and Aggregation**

Venous blood was collected into 1/6 vol acid citrate-dextrose solution (85 mM sodium citrate, 71 mM citric acid, and 9.01 mM dextrose), and platelet-rich plasma was prepared by centrifugation at 100g for 15 minutes at room temperature. Platelets were isolated via differential centrifugation and washing of platelet-rich plasma, using the modified Mustard protocol (Czervionke et al15). The final platelet preparation was suspended in modified Tyrode’s solution (with Ca2+ and Mg2+), and platelet counts, as determined by a Coulter counter (Coulter Corp., Hialeah, Fla.), were adjusted to 5×10^8 cells/ml. Examination of final platelet preparations using Wright’s stain showed an average of two leukocytes (0.05%) per 10,000 platelets.

Platelet aggregation was measured with the usual turbidimetric method.15 Aliquots (50 μl) of serial dilutions of thrombin (0.01-1.0 unit/ml) were added to 450-μl aliquots of platelet suspension stirred at 900 rpm in a dual-channel aggregometer (Payton Scientific, Inc., Buffalo, N.Y.) at 37°C. Changes in light transmittance were recorded for 5 minutes after the addition of thrombin and expressed as percent change. Aggregation responses were quantitated by determining the percent change in light transmittance at 1 and 5 minutes after the addition of 0.1 unit/ml thrombin.

A concentration of 0.1 unit/ml thrombin was used to activate platelets. This concentration of thrombin produces 80–100% of maximal aggregation and does not produce direct vascular effects in rabbit carotid arteries.16 All platelet procedures were carried out at room temperature using plastic or siliconized glassware.

**Serotonin Assay**

Platelets (5×10^8 cells/ml) were activated with thrombin (0.1 unit/ml), and supernatant was collected 5 minutes after the addition of thrombin to allow maximal aggregation and release reaction. Serotonin was extracted from the supernatant with 0.2 M perchloric acid. After centrifugation at 1,600g for 10 minutes, the supernatant was collected and stored in sodium acetate buffer (pH adjusted to 5.0 with sodium hydroxide) at −40°C until assay. Serotonin levels were quantitated by high-performance liquid chromatography with electrochemical detection using previously described chromatographic conditions.17 N-Methyl-5-hydroxytryptamine was used as the internal standard.

**Vessel Preparation**

Normal New Zealand White rabbits (2–3 kg) were anesthetized with sodium pentobarbital (50 mg/kg i.v.), given heparin sodium (150 units/kg i.v.), and killed by exsanguination. Common carotid arteries were removed and immediately placed in cold (5–10°C) oxygenated modified Krebs’ solution containing (mM) NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 · 7H2O 1.2, NaHCO3 25.0, KH2PO4 1.2, and dextrose 11.0. Loose adventitial connective tissue was removed, and one segment 1–1.5 cm long was obtained from each rabbit. Arterial segments were mounted between two plastic cannulas and placed in a 15-ml organ bath.

Carotid arteries were perfused intraluminally and superfused abluminally with oxygenated modified Krebs’ solution by means of a peristaltic pump (Rainin Instrument Co., Woburn, Mass.) at a constant rate of 1.0 ml/min. Pressure at the downstream end of the vessel segment was maintained at 60 mm Hg throughout the experimental protocol. Arterial segments were observed at ×30 magnification, and images were displayed with a video camera attached to the microscope. The outer diameter of the arteries was recorded continuously with an optical system using computer-assisted edge detection and digital analysis of the video image. This preparation has been described in detail previously.16

**Drugs**

Phenylephrine hydrochloride, serotonin creatinine sulfate, acetylcholine hydrochloride, ADP (from equine muscle), sodium nitroprusside, apyrase grade VII (from potato; specific activity, 480 units/mg ATPase, 365 units/mg ADPase, and <0.1 unit/mg AMPase), indomethacin, and Nn-nitro-l-arginine were purchased from Sigma Chemical Co., St. Louis, Mo. Thrombin (bovine) was purchased from Parke-Davis, Morris Plains, N.J. Thromboxane A2 (TXA2) analogue U46,619 was obtained from BIOMOL Research Laboratories, Inc., Plymouth Meeting, Pa. 5-Hydroxytryptamine (5-HT2)–serotonic receptor antagonist LY53,857 was obtained from Research Biochemicals Inc., Natick, Mass. Thromboxane A2/prostaglandin H2 (TXA2/PGH2) receptor antagonist SQ29,548 was provided by E.R. Squibb & Sons, Inc., Princeton, N.J. Phenylephrine, acetylcholine, ADP, LY53,857, apyrase, and sodium nitroprusside were dissolved in deionized water; thrombin was dissolved in sterile water; serotonin was dissolved in 0.1N HCl with 0.1% ascorbic acid; U46,619 and indomethacin were dissolved in ethanol–0.1 M NaHCO3 (1:3 [vol/vol]); and nitro-l-arginine was dissolved in 0.01N HCl to make a stock solution. Each agent was then diluted in modified Krebs’ solution aerated with a mixture of 95% O2–5% CO2 immediately before use. None of the vehicles produced detectable vascular responses. To avoid inactivation of sodium nitroprusside by light, the drug and the stock solution were kept in the dark, and perfusion tubing was covered by aluminum foil.

**Experimental Protocol**

Vessels were perfused for 90 minutes at an intraluminal flow rate of 1.0 ml/min and pressure of 60 mm Hg. Vessel segments were gradually stretched longitudinally to the approximate in situ length and exposed to repeated constrictor (phenylephrine, 10−6 M) and dilator (acetylcholine, 10−4 M) stimuli until reproducible responses were obtained. Endothelial function was evaluated at the beginning and end of the experiment with intraluminal acetylcholine (10−5 M). Acetylcholine produced virtually complete dilatation of phenylephrine-preconstricted arteries. Intraluminal and abluminal administration of unactivated platelets or Tyrodes’ solution, in which platelets were suspended, did not produce detectable changes in vessel diameter. The concentration of thrombin (0.1 unit/ml) that was used to activate platelets did not produce direct vascular effects. For abluminal stimulation, platelet suspensions and drugs were added to the solution bathing the vessels. For intraluminal stimulation, the perfusate was replaced with a solution contain-
ing the desired concentration of platelets or drugs. Platelet suspensions were infused via one limb of the perfusion tubing and were activated intraluminally with thrombin (0.1 unit/ml), which was continuously perfused via the other limb of the perfusion tubing. The length of the perfusion tubing was adjusted to allow maximum activation of platelets with thrombin before reaching the vessel, i.e., approximately 3 minutes at a perfusion rate of 1.0 ml/min. To avoid spontaneous activation of platelets, organ bath and perfusion tubing were siliconized at the beginning of each experiment. Platelet and drug concentrations are reported as final concentrations in the perfusate and bath. Effects of abluminal activation of platelets greater than 5 x 10⁶ cells/ml could not be examined because the vessel image was obscured by the turbidity of platelet suspensions at higher concentrations.

Responses to increasing concentrations of platelets were measured. Vascular responses to each concentration of platelets were recorded for approximately 5–7 minutes until steady-state responses were observed. To examine dilator responses, submaximal tone (EC₅₀) was induced with phenylephrine administered abluminally. Constrictor responses were examined in quiescent vessels. Endothelial function was evaluated with acetylcholine (10⁻⁶ M) and ADP (10⁻⁴ M) immediately after the activation of platelets. Acetylcholine and ADP produced virtually complete dilatation of constricted arteries after activation of normal and hypercholesterolemic platelets. Between each series of interventions, the preparation was washed by changing the bath solution several times with modified Krebs' solution, and an equilibration period of 30 minutes was allowed. Constrictor responses are expressed as percent change in the diameter of quiescent vessels, and dilator responses as percent of preconstriction. The baseline diameter of perfused arteries was 2.5±0.1 mm (mean±SEM).

Responses to activation of platelets and platelet-derived vasoactive agonists were examined in vessels pretreated with 5-HTₐₐ-receptor antagonist LY53,857 (10⁻⁵ M), TXₐₐ/PGL₃ receptor antagonist (10⁻⁵ M), and endothelium-derived relaxing factor (EDRF) synthesis inhibitor Nω-nitro-L-arginine (10⁻⁵ M) applied abluminally for approximately 30 minutes. During intraluminal activation, platelets were activated with thrombin before reaching the vessel, thereby avoiding exposure of platelets to the antagonists before activation. During abluminal application, platelets were added and activated with thrombin immediately after replacing the abluminal perfusate containing the antagonists with fresh Krebs' solution. Specificity and efficacy of each antagonist were tested. We also examined responses to platelets in the presence of apyrase (1.5 units/ml), an enzyme with ADPase and ATPase activity.

In some experiments, platelets were preincubated for 20 minutes with indomethacin (10⁻⁵ M) before activation to examine the role of cyclooxygenase metabolites of the arachidonic acid pathway in mediation of vascular responses to platelets.

**Statistical Analysis**

Data are presented as mean±SEM. Student's t test was used for statistical analysis of differences between paired and unpaired data. A value of p<0.05 was considered statistically significant. None of the data in this study has been described in other publications.

**Results**

**Baseline Data**

Total and low density lipoprotein cholesterol levels were higher in hypercholesterolemic patients than in healthy control subjects (Table 1). Plasma triglycerides and high density lipoprotein cholesterol were similar in the two groups. All subjects had normal hematological and plasma biochemical values, except for lipid variables (data not shown).

**Platelet Aggregation and Release of Serotonin**

Platelet aggregation was greater (p<0.05) in hypercholesterolemic patients (68±3% and 93±2% change in light transmittance at 1 and 5 minutes, respectively; n=8) than in control subjects (41±3% and 83±2% change in light transmittance at 1 and 5 minutes, respectively; n=8). Release of serotonin from activated platelets (5 x 10⁶/ml) was significantly greater (p<0.05) in hypercholesterolemic patients (310±57 nM, n=8) than in normal subjects (82±13 nM, n=8).

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Recordings of arterial diameter during intraluminal activation of platelets from a normal subject (left panel) and hypercholesterolemic patient (right panel). The arteries were constricted with 10⁻⁵ M phenylephrine, and platelets were activated with 0.1 unit/ml thrombin.
Response to Platelets

Constricted arteries. Intraluminal activation of platelets from normal subjects produced profound vasodilatation (Figures 1 and 2). Vasodilator responses to intraluminal activation of platelets from hypercholesterolemic patients were significantly impaired (Figures 1 and 2).

Quiescent arteries. Abluminal activation of platelets produced modest constriction (Figure 3). Vasodilator responses to abluminal activation of platelets were augmented in hypercholesterolemic patients.

Effect of Inhibitors on Response to Platelets

Baseline (quiescent) arterial diameter was not affected by LY53,857, SQ29,548, nitro-L-arginine, or apyrase. Constricted arteries. Pretreatment with LY53,857 (10⁻⁵ M) augmented vasodilator responses to both normal and hypercholesterolemic platelets, but the effect was quite small (Figure 4). However, LY53,857 failed to restore dilator responses to hypercholesterolemic platelets to normal. SQ29,548 (10⁻³ M) had no detectable effect on dilator responses to platelets. The combination of LY53,857 (10⁻³ M) and SQ29,548 (10⁻³ M) had no additional effect on dilator responses to platelets beyond the effect of LY53,857 alone (Figure 4).

Nitro-L-arginine (10⁻³ M) inhibited vasodilatation produced by intraluminal activation of both normal and hypercholesterolemic platelets and ADP (Figure 5). Dilator responses to sodium nitroprusside were not affected by nitro-L-arginine (Figure 5).

Apyrase virtually abolished vasodilatation produced by intraluminal activation of both normal and hypercholesterolemic platelets (Figure 6). Dilator responses to ADP, but not acetycholine, were inhibited by apyrase (Figure 6).

Pretreatment of platelets with indomethacin (10⁻⁵ M) had no significant effect on dilator responses to normal (n=5) and hypercholesterolemic (n=3) platelets. Vasodilator responses to normal platelets (1x10⁶ platelets/ml) were 28±7% and 27±4% before and after pretreatment with indomethacin, respectively, and responses to hypercholesterolemic platelets were 8±2% and 6±1% before and after pretreatment with indomethacin, respectively.

Quiescent arteries. LY53,857 (10⁻⁵ M) abolished constrictor responses to the abluminal activation of platelets and serotonin but not to U46,619 (Figure 7). SQ29,548 (10⁻³ M) abolished constrictor responses to U46,619 but had no significant effect on platelet- and serotonin-induced vasoconstriction (Figure 7).

In arteries pretreated with nitro-L-arginine, intraluminal activation of 2.5x10⁶ platelets/ml produced modest vasoconstriction. The response to platelets from hypercholesterolemic patients (-17±2%, n=5) was significantly greater than the response to platelets from normal subjects (-5±1%, n=5).

In the presence of apyrase, intraluminal activation of 2.5x10⁶ platelets/ml produced modest vasoconstriction. In the presence of apyrase, the vasodilator response to platelets from hypercholesterolemic patients (-15±3%, n=3) was significantly greater than the response to platelets from normal subjects (-6±2%, n=5).

Discussion

The major finding in this study is that vasodilator responses to intraluminal activation of platelets from hypercholesterolemic patients are profoundly impaired, and vasodilator responses to platelets are augmented in hypercholesterolemic patients. Previous studies have demonstrated altered platelet function in type II hypercholesterolemia. The findings in the present study indicate that abnormalities in platelet function in hypercholesterolemia are of sufficient magnitude to result in profoundly altered vas-
cular responses. Thus, we suggest that altered platelet function, as well as altered vascular reactivity, may contribute to altered vascular responses to platelets in hypercholesterolemia and atherosclerosis.

**Platelet Function in Hypercholesterolemia**

Several studies have shown that platelet function is altered in hypercholesterolemic humans and animals. There is an increase in platelet adhesion, aggregation, and release of serotonin, adenine nucleotides, platelet factors 3 and 4, and β-thromboglobulin. There also appears to be augmented arachidonic acid metabolism, with increased production of TxA₂ in hypercholesterolemia. Decreased sensitivity to prostacyclin (prostaglandin I₂) has also been reported in hypercholesterolemia.

**Vascular Responses to Platelets**

Vascular responses to the activation of platelets are a balance of vasodilator activity mediated primarily by adenine nucleotides and vasoconstrictor activity mediated by serotonin and thromboxane. Activated normal human platelets release almost 20-fold more adenine nucleotides than serotonin and approximately 600-fold more adenine nucleotides than thromboxane. Thus, the predominant response to activation of normal human platelets is vasodilatation.

**Constricted arteries.** In the present study, intraluminal activation of platelets produced dilatation in perfused carotid arteries preconstricted with phenylephrine. These results confirm our previous findings that intraluminal ADP and ATP produce dilatation in perfused carotid arteries. The difference in vascular responses to intraluminal and abuminal activation of human platelets may be attributed to the absence of vasodilatation in response to abuminal ADP, which is the major vasodilator product released by human platelets.

In a recent study, we suggested that asymmetric vascular responsiveness to ADP is due primarily to preferential activation of P₂X purinoceptors on smooth muscle and P₂Y purinoceptors on endothelium. During intraluminal administration of ADP, endothelium-dependent vasodilatation dominates over direct endothelium-independent vasoconstriction. During abluminal administration, ADP reaches the medial P₂X purinoceptor located on endothelium, thereby favoring constriction.

**Quiescent arteries.** Abluminal activation of platelets produced constriction in quiescent carotid arteries. Vasocostractive responses to platelets were virtually abolished by 5-HT₂-serotonergic antagonist LY53,857 and not by TxA₂/PGF₂α receptor antagonist SQ29,548. These results are concordant with our previous findings that constractor responses to human platelets in rabbit carotid arteries are mediated primarily by activation of 5-HT₂-serotonergic receptors.

**Mechanism of Altered Vascular Responses to Platelets From Hypercholesterolemic Humans**

**Constricted arteries.** Impaired vasodilator responses to intraluminal administration of hypercholesterolemic platelets could be due to either the increased release of the vasoconstrictors serotonin and thromboxane or the decreased release of the vasodilator ADP. Release of ADP from hypercholesterolemic platelets, however, is not reduced. In fact, previous studies indicate that platelets from hypercholesterolemic patients release increased amounts of ADP, as well as serotonin and thromboxane.

Despite an increased release of serotonin from activated platelets in hypercholesterolemic patients, as shown in this and previous studies, 5-HT₂-serotonergic antagonist LY53,857 failed to restore vasodilator responses to hypercholesterolemic platelets to normal. This finding suggests that increased release of

**Figure 4.** Bar graphs showing the effect of 5-hydroxytryptamine, antagonist LY53,857 (LY, 10⁻⁵ M) and thromboxane A₂/prostaglandin H₂ receptor antagonist SQ29,548 (SQ, 10⁻³ M) on responses to intraluminal activation of platelets (2.5x10⁸ platelets/ml) from normal subjects (N, n=5) and hypercholesterolemic patients (HC, n=5). Values (mean±SEM) are percent change in diameter from a preconstricted diameter of 1.7±0.2 mm. *p≤0.05 vs. control (Con).

**Figure 5.** Bar graphs showing the effects of nitric-arginine (N-arg, 10⁻⁵ M) on responses to intraluminal activation of platelets (2.5x10⁸ platelets/ml) from normal subjects (N, n=5) and hypercholesterolemic patients (HC, n=5) and ADP (10⁻³ M) and sodium nitroprusside (SNP, 10⁻³ M) in phenylephrine-preconstricted arteries. Values (mean±SEM) are percent change in diameter from a preconstricted diameter of 1.8±0.2 mm. *p≤0.05 vs. control (Con).
serotonin may not contribute importantly to impaired dilator responses to hypercholesterolemic platelets.

Increased arachidonic acid metabolism with augmented thromboxane production has been reported in response to agonist-induced activation of platelets in hypercholesterolemia. In this study, cyclooxygenase inhibitor indomethacin and TXA2/PGH2 receptor antagonist SQ29,548 had no effect on vascular responses to platelets from normal subjects and hypercholesterolemic patients. TXA2, released by activated human platelets, does not contribute to platelet-mediated constrictor responses in rabbit carotid arteries. Therefore, it is unlikely that thromboxane contributes significantly to altered responses of rabbit carotid arteries to platelets from hypercholesterolemic patients.

Apyrase and nitro-L-arginine abolished the dilatation produced by intraluminal activation of both normal and hypercholesterolemic platelets in preconstricted arteries. We considered the possibility that, if hypercholesterolemic platelets release large amounts of ADP, the direct constrictor effect of ADP on vascular smooth muscle might mask the vasodilator response to EDRF that is released by ADP. On the basis of the effects of apyrase, however, it seems unlikely that the release of increased amounts of ADP from hypercholesterolemic platelets accounts for impaired vasodilatation in the present study.

We have considered the possibility that vasodilator responses to hypercholesterolemic platelets were impaired in this preparation because the platelets were hyperaggregable, and thus may have released their vasoactive products earlier than normal platelets, before reaching the vessel in which responses were examined. The half-life of serotonin and thromboxane, which are the vasoconstrictor products released by platelets, is shorter (60–120 seconds and 30 seconds, respectively) than the half-life of ADP (4 minutes). Thus, if hypercholesterolemic platelets released their vasoactive products earlier than normal platelets, one would anticipate that dilator responses to hypercholesterolemic platelets would have been greater, because the concentration of serotonin and thromboxane would have been reduced more than the concentration of ADP. It is unlikely, therefore, that increased aggregability of hypercholesterolemic platelets accounts for the finding that vasodilator responses are impaired.

Failure to restore responses to hypercholesterolemic platelets to normal by the serotonin and thromboxane antagonists and apyrase indicates that mechanisms in addition to augmented release of serotonin, thromboxane, and ADP contribute to impaired vasodilator responses to hypercholesterolemic platelets. We speculate that several potential mechanisms may play a role in altered vascular responses to platelets in hypercholesterolemia. First, platelets have been shown to produce oxygen-derived free radicals. Oxygen radicals are generated during arachidonic acid metabolism in platelets, and because arachidonic metabolism is increased in hypercholesterolemic platelets, generation of oxygen radicals may be increased. Oxygen radicals impair endothelium-dependent relaxation by inactivating EDRF and oxygen radicals may also potentiate constrictor responses. Thus, we speculate that oxygen-derived free radicals released by hypercholesterolemic platelets may inactivate EDRF, impair dilator responses, and contribute to excessive vasoconstriction.

Second, hypercholesterolemic platelets may release increased amounts of platelet-derived growth factor, a potent vasoconstrictor or other vasoconstrictor substances. We know of no evidence to support this possibility. Third, activated platelets release cholesterol, either as surface-bound low density lipoprotein or as platelet factor 3. Low density lipoprotein has been reported to impair endothelium-dependent dilatation, probably by inactivating EDRF. In addition, low density lipoprotein that is modified by products of arachidonic acid metabolism, such as malondialdehyde or free radicals, may act directly on

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**Figure 6.** Bar graphs showing the effects of apyrase (1.5 units/ml) on responses to intraluminal activation of platelets (2.5×10⁷ platelets/ml) from normal subjects (N, n=5) and hypercholesterolemic patients (HC, n=3) and ADP (10⁻⁵ M) and acetylcholine (ACH, 10⁻⁵ M) in phenylephrine-preconstricted arteries. Values (mean±SEM) are percent change in diameter from a preconstricted diameter of 1.7±0.2 mm. *p≤0.05 vs. control (Con).

**Figure 7.** Bar graphs showing the effects of thromboxane A2/prostaglandin H2 receptor antagonist SQ29,548 (SQ, 10⁻⁷ M) and 5-hydroxytryptamine2 antagonist LY53,857 (LY, 10⁻⁵ M) on responses to abluminal activation of platelets (5×10⁷ platelets/ml) from normal subjects (N, n=5) and hypercholesterolemic patients (HC, n=5) and 5-hydroxytryptamine (serotonin, 10⁻⁵ M) and thromboxane A2 analogue U46,619 (10⁻⁵ M) on quiescent arteries. Values (mean±SEM) are percent change in diameter from a quiescent diameter of 2.4±0.1 mm. *p≤0.05 vs. control (Con).
vascular smooth muscle to potentiate vasoconstriction. Third, hypercholesterolemic platelets may release substances that could induce the ADPase activity of endothelium. Thus, augmented ADPase activity of endothelium could contribute to impaired vasodilator responses to hypercholesterolemic platelets. We know of no evidence to support this possibility.

Quiescent arteries. More serotonin was released by platelets from hypercholesterolemia patients than by platelets from normal subjects, and vasoconstrictor responses to abluminal application of platelets from hypercholesterolemic patients were virtually abolished by the 5-HT, antagonist LY53,857. Thus, increased vasoconstrictor responses to abluminal application of platelets from hypercholesterolemic patients may be mediated primarily by increased release of serotonin.

Implications

Atherosclerosis alters vascular responses to vasoactive factors released by platelets. Previous studies have focused on mechanisms by which abnormalities of the vessel wall, especially the endothelium, lead to abnormal responses. The new finding in this study is that alteration of platelets by hypercholesterolemia may also contribute to abnormal vascular responses. Thus, we speculate that endothelial dysfunction, together with alteration of platelets by hypercholesterolemia, may predispose to susceptibility to vasospasm in atherosclerosis.

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