Endogenous Nitric Oxide and Prostaglandins Synergistically Counteract Thromboembolism in Arterioles but Not in Venules

Martijn A.W. Broeders, Geert-Jan Tangelder, Dick W. Slaaf, Robert S. Reneman, Mirjam G.A. oude Egbrink

Abstract—It has been shown that NO and prostacyclin (prostaglandin I₂) from cultured endothelium synergistically inhibit blood platelet aggregation in vitro. However, it is unknown whether this synergism is also effective in the inhibition of thromboembolism in vivo, and, if it is, whether it differs between vessel types. Therefore, the effect of endogenous NO and prostacyclin, in combination or alone, on thromboembolism was studied in an in vivo model. Thromboembolism was induced by micropipette puncture of rabbit mesenteric arterioles and venules (diameter 18 to 40 μm). In addition, the influence of wall shear rate was analyzed. In arterioles, the combined inhibition of NO synthase (N⁶-nitro-L-arginine [L-NA] 0.1 mmol/L; local superfusion) and of cyclooxygenase (aspirin [ASA] 100 mg/kg IV) resulted in a pronounced, significant prolongation of embolization duration (median >600 seconds) compared with control (median 153 seconds). This combined effect of L-NA + ASA was greater than the sum of the individual effects of L-NA and ASA. In contrast, in venules L-NA + ASA had no additional effect on embolization duration (209 seconds) compared with the effect of L-NA alone (230 seconds); ASA alone had no effect (122 seconds; control 72 seconds). Interestingly, only in the L-NA + ASA arterioles did embolization correlate positively with wall shear rate (rₛ = 0.687; P = 0.028). In conclusion, this study indicates that in arterioles, but not in venules, endogenous NO and prostaglandins synergistically counteract ongoing thromboembolism after vessel wall injury and that the combination of endogenous NO and prostaglandins appears to protect against enhancement of arteriolar thromboembolism by wall shear rate. (Arterioscler Thromb Vasc Biol. 2001;21:163-169.)

Key Words: vessel wall injury ■ thromboembolism pathophysiology ■ nitric oxide ■ prostaglandins ■ wall shear rate

After vessel wall injury, platelets are activated and a platelet thrombus is formed. To limit the extent of platelet activation, the vessel wall releases potent platelet-inhibiting substances such as prostacyclin (prostaglandin [PG]I₂) and NO. Better insight into the role of endogenous antithromboembolic substances is of major importance to improve our understanding of clinical disorders in which thromboembolic processes play a role. Clinical studies indicate that atheromatous changes in the arterial wall are preceded by macrovascular¹,² and microvascular³,⁴ “endothelial dysfunction.” It has been established that reduced bioavailabilities of vascular NO and PGI₂ are key factors in this dysfunction,⁵ which may be reversed by stimulating the endogenous production of NO⁶ or PGI₂.⁵

In 2 separate in vivo studies, we investigated the individual roles of endogenous prostaglandins and NO in inhibition of thromboembolism in arterioles and venules.⁶,⁷ From these studies, we concluded that in arterioles, this process is predominantly inhibited by endogenous prostaglandins,⁶ whereas inhibition by endogenous NO is more important in venules.⁷ These antithromboembolic mediators can be produced simultaneously by vascular endothelium at a site of wall injury, and their production is stimulated by platelet agonists such as thrombin and ADP.⁸,⁹ Therefore, in injured vessels, activated blood platelets are likely to be exposed to a combination of the platelet antagonists PGI₂ and NO. This combination may exert a more pronounced antithromboembolic effect than expected on the basis of their individual antithromboembolic effects, because in vitro studies have shown that PGI₂ and NO released by cultured endothelial cells can act synergistically in the inhibition of platelet aggregation.¹⁰ It is unknown, however, whether this synergistic interaction is also effective in the inhibition of thromboembolism in vivo and, if it is, whether it differs between vessel types.

The aim of the present study was to investigate in vivo the effect of the combined inhibition of endogenous prostaglandin and NO production on thromboembolism in arterioles and

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venules by using a model described in detail previously. In anesthetized rabbits, the wall of mesenteric arterioles and venules is punctured with a micropipette, and the ensuing thromboembolic reaction is studied with the use of intravital videomicroscopy. To study the combined effects of endogenous prostaglandins and NO on this thromboembolic reaction, rabbits were pretreated with intravenous aspirin (ASA) and their mesentery was superfused with the NO synthase inhibitor NG-nitro-L-arginine (L-NA) before wall puncture. In separate experiments, rabbits were treated with ASA or L-NA alone or served as controls (CON group).

Methods

Animals and Intravital Video Microscopy

Experiments were performed on 27 New Zealand White rabbits of either sex (weight from 2.3 to 2.8 kg). The experiments were approved by the local ethics committee on the use of laboratory animals. Anesthesia was induced with ketamine hydrochloride (40 mg/kg body wt Nimatek IM; A.U.V.) and xylazine hydrochloride (5 mg/kg body wt Sedanum IM; A.U.V.). Anesthesia was maintained by the continuous infusion of ketamine (40 mg/kg·h−1), xylazine (5 mg/kg·h−1), and lactetrol (15 mL/h; Aesculaap) through a catheter (PE-50) in the femoral vein. Arterial blood pressure was continuously measured through a catheter (PE-60) in the femoral artery (Uniflow external pressure transducer; Baxter). Heart rate was assessed from the instantaneous pressure signal. To keep the arterial catheter patent, it was continuously perfused with physiological saline (3 mL/h) via the Uniflow system; no heparin was used. Arterial pressure and heart rate values were stored on hard disk.

During surgery and throughout the experiment, body temperature was maintained at 37° to 38°C with an infrared heating lamp maintained at 37° to 38°C with an infrared heating lamp (Uniflow external pressure transducer; Baxter). Heart rate was assessed from the instantaneous pressure signal. To keep the arterial catheter patent, it was continuously perfused with physiological saline (3 mL/h) via the Uniflow system; no heparin was used. Arterial pressure and heart rate values were stored on hard disk.

During surgery and throughout the experiment, body temperature was maintained at 37° to 38°C with an infrared heating lamp controlled by a thermoanalyzer system (Hugo Sachs Elektronik) connected to a rectal probe. After surgery, blood from a central ear artery was collected in EDTA (0.9 mL blood/0.1 mL EDTA; 0.027 mol/L) for electronic platelet counts and assessment of hemoglobin concentration and hematocrit (model ZF; Coulter Counter); these values were corrected for EDTA dilution.

During the experiments, the rabbits were ventilated (animal ventilator model 4601; Technical & Scientific Equipment) at a positive end-expiratory pressure of ≈2 cm H2O through a trachea cannula (3.5 or 4.5 mm ID; Mallinckrodt) with a mixture of nitrogen (75%), oxygen (24.5%), and carbon dioxide (0.5%) to maintain arterial saturation of 100% and PaO2 of 100 ± 10 mm Hg. In short, all points in time that an embolus broke off the stationary thrombus were determined (time of vessel wall puncture: t = 0). The time needed to produce an embolus was defined as the interval between puncture and the breaking off of the first embolus or as the interval between the breaking off of successive emboli. Per vessel, the median of these intervals was determined, representing the median embolus production time for that vessel. Each vessel was observed continuously for 600 seconds from the moment of puncture. Vessels in which embolization continued for >600 seconds were observed again intermittently every 15 minutes, for 1 to 2 minutes, during the remainder of the experiment; therefore, in these vessels, the total duration of embolization could only be approximated. Emboli were taken into account only when their short axis, perpendicular to the vessel wall, was >5 μm. Aggregates of smaller dimensions could not always be distinguished from the background with sufficient accuracy. To increase the number of blood vessels in each group, we punctured several vessels per rabbit mesentery.

Experimental Groups and Protocol

Rabbits were assigned at random to 1 of 4 groups. In the group of rabbits that is most important for this study (n=8), both endogenous NO and endogenous prostaglandin synthesis was inhibited (L-NA + ASA group). Rabbits in this group were pretreated with ASA (100 mg/kg IV), whereas their mesentery was continuously superfused with the NO synthase inhibitor L-NA (0.1 mmol/L; molecular weight 219.2; Sigma Chemical Co). L-NA is an irreversible inhibitor of NO synthase, and a concentration of 0.1 mmol/L has been shown to be sufficiently high to effectively inhibit NO synthase in the wall of rabbit blood vessels and in rabbit platelets. ASA at a dose of 100 mg/kg inhibits not only the endogenous production of the proaggregatory thromboxane A2, but also that of the antiaggregatory prostaglandins (eg, PGI2) for at least the duration of our experiments. Aspirin was administered through a polyethylene catheter in the marginal ear vein. High doses of ASA irreversibly acetylate the active site of the prostaglandin-synthesizing enzyme cyclooxygenase (COX).

In 2 additional groups of rabbits, the role of endogenous NO (L-NA group, n = 6) and endogenous prostaglandins (ASA group, n = 7) was studied separately. The inhibition of endogenous NO and endogenous prostaglandin production was performed according to the same methods as used in the L-NA + ASA group. In the CON group (n = 6), the mesentery was superfused with the vehicle, a buffered Tyrode’s solution. The experiments in these 3 groups were newly performed and not used before in previous studies. Such control experiments have to be performed with rabbits from the

Vessel Wall Puncture and the Thromboembolic Reaction

Arterioles and venules with an estimated diameter of 20 to 40 μm were selected. Vessel wall injury was mechanically induced by puncture with a glass micropipette (tip diameter ≈6 μm), as described previously. To be certain that all layers of the wall were damaged, puncture was considered to be successful only if red blood cells could be seen leaving the vessel.

Immediately after puncture, the thromboembolic reaction started. In all vessels, a white thrombus was formed that consisted of tightly packed platelets. The height and shape of the thrombus remained constant in time. Circulating platelets adhered to this stationary thrombus mainly on its downstream side, forming a loosely packed platelet mass that did not affect the height of the stationary thrombus. From time to time, these platelet masses embolized. After a certain period of time, embolization stopped in most vessels, whereas the thrombus remained unchanged at the site of injury for the remainder of the experiment. Only in a minority of vessels (2 of all 49 venules; none of the arterioles) did bleeding and thrombus formation occur without the subsequent production of emboli. In these 2 vessels, no distinguishable emboli were produced.

To quantify the thromboembolic reaction, the following variables were determined offline from videotape: the duration of bleeding (bleeding time), the maximal thrombus height relative to the local vessel diameter, the duration of embolization, the number of emboli produced, and as a measure of the rate of embolus formation, the median embolus production time per vessel (ie, the median of all periods of time needed to produce an embolus). In short, all points in time that an embolus broke off the stationary thrombus were determined (time of vessel wall puncture: t = 0). The time needed to produce an embolus was defined as the interval between puncture and the breaking off of the first embolus or as the interval between the breaking off of successive emboli. Per vessel, the median of these intervals was determined, representing the median embolus production time for that vessel. Each vessel was observed continuously for 600 seconds from the moment of puncture. Vessels in which embolization continued for >600 seconds were observed again intermittently every 15 minutes, for 1 to 2 minutes, during the remainder of the experiment; therefore, in these vessels, the total duration of embolization could only be approximated. Emboli were taken into account only when their short axis, perpendicular to the vessel wall, was >5 μm. Aggregates of smaller dimensions could not always be distinguished from the background with sufficient accuracy. To increase the number of blood vessels in each group, we punctured several vessels per rabbit mesentery.
same population and within the same period of time to prevent an increase in variability due to seasonal influences.

Both the L-NA– and ASA-containing solutions were freshly prepared on the day of each experiment. L-NA was dissolved in buffered Tyrode’s solution and ASA was suspended in water at a concentration of 100 mg/mL as previously described.6,7 The ASA solution was administered intravenously at a rate of 1 mL/min. In a previous study, we showed that the vehicle solution (with the same pH) did not affect thromboembolism when administered intravenously.6

In all groups, the mesentery was allowed to stabilize for a period of 30 to 35 minutes after exteriorization under continuous superfusion with buffered Tyrode’s solution. Fifteen minutes before the start of this stabilization period, the rabbits in the ASA and L-NA + ASA groups were pretreated with ASA, as described earlier. After the stabilization period, the superfusion was switched to the L-NA solution in the L-NA and L-NA + ASA groups; in the CON and ASA groups, superfusion was continued with buffered Tyrode’s solution. Per experiment, a median number of 4 vessels (range 1 to 6 vessels) were punctured from 30 to 35 minutes up to 3 hours after exteriorization of the mesentery. Each puncture was preceded by a 4-minute period during which mean red blood cell velocity was measured. This 4-minute period, the puncture itself, and the subsequent observation period of ≥600 seconds were recorded on videotape.

Statistical Analysis
Because of their nonsymmetrical distribution, the data are presented and displayed as median values with interquartile ranges unless otherwise indicated. Embolization data are presented per blood vessel. Puncture of different numbers of vessels per rabbit in the various groups did not influence interpretation of the data, because averaging of data per animal led to similar results and conclusions. Differences between 2 groups were tested with the nonparametric Mann-Whitney U test. Correlations were performed with the nonparametric Spearman rank correlation test (coefficient rs). In all tests, the level of significance was set at 5%.

Endogenous NO and prostaglandins were considered to act synergistically when the effect of inhibition of both was greater than the sum of the effects of inhibition of NO (by L-NA) and prostaglandins (by ASA) alone; this definition of synergism has been used by others.19

Results
The Thromboembolic Reaction In Vivo
In all vessels, bleeding and thrombus formation started immediately after wall puncture. Bleeding duration did not differ between the groups (Table 1). A thrombus started to grow within 0.1 second after puncture and reached its maximal size within 1 to 2 seconds. This fast thrombus formation, as well as the height of the thrombus (Table 1), was not influenced by combined L-NA and ASA treatment or treatment with L-NA or ASA alone, in both arterioles and venules. In both the L-NA and ASA groups, no significant correlation was found between the duration of the experiment until a vessel was punctured and the embolization process in that vessel, indicating that there was no time dependency of the embolization parameters measured and that L-NA and ASA treatment were effective throughout the experiments.

Arterioles
In these vessels, the combination of L-NA and ASA resulted in a pronounced, significant prolongation of the duration of embolization (median >600 seconds) compared with each of the other 3 groups (CON 153 seconds, L-NA 234 seconds, ASA 314 seconds; all P<0.008; Figure 1A). It should be noted that the median value of 600 seconds is an underestimation of the combined effect of L-NA and ASA due to the finite observation period. In the 70% of the arterioles of this group in which embolization continued at >600 seconds, it continued during the remainder of the experiment (30 minutes to 3 hours). L-NA alone had no significant effect, whereas ASA pretreatment induced a significant prolongation compared with CON. The latter effect, however, was significantly less than the effect of combined L-NA and ASA treatment. Moreover, in only 21% of the ASA arterioles did embolization continue for >600 seconds, which also shows that the effect of ASA alone is clearly less than the combined effect of L-NA + ASA.

The number of emboli produced per vessel within 600 seconds (Table 1) was also significantly larger in the L-NA + ASA group (29 emboli) than in the other 3 groups (CON 14, L-NA 16, ASA 12).

### Table 1. Thromboembolic Parameters and Bleeding Times in Arterioles and Venules of the 4 Experimental Groups

<table>
<thead>
<tr>
<th></th>
<th>Arterioles</th>
<th>Venules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>L-NA</td>
</tr>
<tr>
<td>Bleeding time, s</td>
<td>1.2 (0.4–13.9)</td>
<td>0.5 (0.1–5.3)</td>
</tr>
<tr>
<td>Th, %</td>
<td>74 (28–85)</td>
<td>71 (49–88)</td>
</tr>
<tr>
<td>Emboli</td>
<td>14 (5–34)</td>
<td>16 (3–52)</td>
</tr>
<tr>
<td>EPT, s</td>
<td>12 (6–23)</td>
<td>11 (4–19)</td>
</tr>
</tbody>
</table>

Bleeding time indicates the initial bleeding duration; Th, relative thrombus height; Emboli, number of emboli produced within 600 seconds after puncture; and EPT, median embolus production time, a measure of the rate of embolus formation. Median values and (ranges) are presented.

*P<0.05 compared with each of the other groups.
†P<0.01 compared with CON.

Figure 1. The effect of inhibition of NO synthase (NOS) by L-NA, of COX by ASA, or of both (L-NA + ASA) on the duration of embolization in arterioles (left) and venules (right). Data obtained in CON group (CON group: 8 arterioles, 11 venules), L-NA group (11 arterioles, 12 venules), ASA group (19 arterioles, 14 venules), and L-NA + ASA group (10 arterioles, 12 venules) are presented as median values (●) with their interquartile ranges (vertical lines). *P<0.05, **P<0.01, and ***P<0.001.
The median embolus production time, as a measure of the rate of embolus production (Table 1), was not significantly influenced by L-NA + ASA or by L-NA or ASA alone.

**Venules**

In venules, combined treatment with L-NA and ASA resulted in a significant, but far less pronounced, prolongation of embolization duration (L-NA + ASA 209 seconds; Figure 1B) compared with CON (72 seconds); this prolongation was not significantly different from the increase observed after L-NA treatment alone (230 seconds) but was significantly greater than the increase after ASA pretreatment alone (122 seconds). The increase in duration of embolization after L-NA superfusion was significant compared with the CON group. In only 1 of the 49 venules did embolization continue for longer than the 600-second observation period.

Compared with the CON group (3 emboli), the number of emboli produced (Table 1) was not significantly changed by combined L-NA and ASA treatment (8 emboli), whereas L-NA superfusion alone significantly increased the number of emboli (10 emboli). ASA pretreatment alone had no significant effect (5 emboli).

The median embolus production time (Table 1) in the L-NA + ASA group was not significantly different compared with the CON, ASA, and L-NA groups.

**Synergism in Arterioles and Venules?**

In arterioles, the median effect of a combination of L-NA and ASA on embolization duration (>600 seconds, which is >447 seconds longer than in the CON group [153 seconds]) and on the number of emboli produced (>29 emboli, which is an increase of ≥15 emboli compared with the CON group [14 emboli]) was greater than the sum of the median effects of L-NA (234 seconds, which is 81 seconds longer than the CON group; 16 emboli, which is an increase of 2 emboli) and ASA (314 seconds, which is 161 seconds longer than the CON group; 12 emboli, which is a decrease of 2 emboli) alone. Hence, synergy is present because 447 seconds is longer than 242 (81 + 161) seconds and 15 emboli is >0 ([1 + 2] + [−2]) emboli. In contrast, no such synergistic effect of the combined inhibition of NO synthase and COX was found in venules.

**Arterioles Versus Venules**

In the CON, ASA, and L-NA + ASA groups, the duration of embolization and the number of emboli produced were significantly larger in arterioles than in venules. In contrast, in the L-NA group, the embolization parameters were similar in arterioles and venules (all \( P > 0.218 \)).

**Fluid Dynamic Conditions**

The fluid dynamic parameters as measured in all vessels immediately before puncture are presented in Table 2. Neither L-NA + ASA nor L-NA or ASA alone had a significant effect on diameter, red blood cell velocity, and reduced velocity in the venules. Except for a slightly lower red blood cell velocity and reduced velocity in the L-NA + ASA arterioles than in the L-NA arterioles (\( P = 0.049 \)), the fluid dynamic parameters were similar in the arterioles of the 4 groups.

In the L-NA + ASA arterioles, reduced velocity, which is a measure of wall shear rate, correlated positively with the number of emboli produced within 600 seconds (\( r_s = 0.687, P = 0.028 \); Figure 2, arterioles). In contrast, in all arterioles of the other 3 groups (all \( r_s < 0.209 \), all \( P > 0.214 \); data not shown), as well as in the venules of all groups (all \( r_s < 0.251 \), all \( P > 0.092 \); data not shown), no correlation was found between reduced velocity and the embolization parameters.

**Whole-Animal Parameters**

In all rabbits, the values of hemoglobin (overall median value 7.9 mmol/L, range 7.0 to 9.6 mmol/L), hematocrit (38.2 L/L, 32.4 to 45.8 L/L), platelet count (548 \( \times 10^3 \)/μL, 384 to 978 \( \times 10^3 \)/μL), mean arterial blood pressure (69 mm Hg, 53 to 102 mm Hg), and heart rate (142 bpm, 115 to 172 bpm) were within the ranges normally found in anesthetized rabbits. 11, 12

Arterial blood pressure and heart rate did not change after ASA pretreatment or L-NA superfusion. No significant differences in hemoglobin, hematocrit, platelet counts, mean arterial blood pressure, and heart rate were found between rabbits of the different groups. In addition, no significant correlations were found between these whole-animal parameters and the embolization parameters in arterioles and venules.

![Figure 2](http://atvb.ahajournals.org/Downloaded_from/.../7_4_2017.jpg)

**Figure 2**. Number of emboli produced during the 600-second period of observation in the arterioles (left) and venules (right) of the L-NA + ASA group as a function of reduced velocity \( (U = \text{mean red blood cell velocity/diameter}) \), which is a measure of wall shear rate. Note that the range of the number of emboli produced differs between arterioles and venules.
Discussion

The findings in the present study show that combined inhibition of endogenous NO and prostaglandin production affects the thromboembolic reaction after wall puncture differently in arterioles and venules of the rabbit mesentery. In arterioles, the combined inhibition of endogenous NO and prostaglandin production with L-NA and ASA results in a prolongation of the duration of embolization and a concomitant increase in the number of emboli produced that was greater than the sum of the individual effects of L-NA and ASA. Such a synergistic effect was not observed in venules. In addition, this combined inhibition unmasked a positive relation between embolization and wall shear rate in arterioles but not in venules.

The present study shows that the inhibition of endogenous NO and prostaglandin formation only influences embolization but has no effect on thrombus formation. In both arterioles and venules, wall puncture induces a thromboembolic reaction that consists of these 2 stages. First, a stable thrombus is formed by heavily activated blood platelets that are tightly packed and completely degranulated and shape changed. Subsequently, emboli are produced at the downstream side of this thrombus. Platelets in the emboli are loosely packed without clear signs of shape change or degranulation. Hence, the level of platelet activation appears to be clearly different during thrombus formation and embolization. Therefore, it is not surprising that platelet-influencing substances like PGI2 and NO exert different actions during thrombus formation and embolization.

Wall shear rate has been shown to be a platelet-stimulating factor in vitro and in damaged arteries in vivo. In intact vessels in vivo, however, shear forces also stimulate the endothelial production of such platelet-inhibiting factors as NO and PGI2. Therefore, shear forces may have only a stimulating effect on thromboembolism in vivo after both vessel wall damage and complete inhibition of endothelium-derived antiplatelet factors. In the present study, we demonstrate for the first time that in arterioles, wall shear rate enhances the thromboembolic reaction only when the endogenous production of both NO and prostaglandins is blocked. This finding indicates that under normal circumstances, these autacoids play an important role in counteracting wall shear rate–enhanced platelet activation in arterioles.

In venules, platelet-vessel wall interactions are reported to be shear dependent, too. Because in our study the parameters that describe these interactions are independent of wall shear rate, even when both endogenous NO and prostaglandin synthesis is blocked, other factors that oppose a wall shear rate–enhanced platelet activation have to be considered. In this respect, ectonucleotidases, anticoagulant agents, or fibrinolytic substances should be taken into account.

An additional possible element in the marked effect of wall shear rate on embolization in arterioles when both endogenous NO and prostaglandin synthesis are blocked is that unlike in venules, the balance between anti thromboembolic and prothromboembolic properties of the wall is more toward prothromboembolic. This is supported by our repeated finding that under control conditions, the duration of embolization is significantly longer in arterioles than in venules.

The present study presents for the first time a pronounced synergistic effect of endogenous NO and prostaglandins on thromboembolism in vivo in arterioles but not in venules. The combined action of both substances has been studied before with regard to vascular reactivity, either with isolated human arteries and veins or in vivo in hamster arterioles and rat arterioles and venules, but not with regard to their antithromboembolic function. Several reports describe the combined effects of exogenously added NO and PGI2 on platelet function in vivo (or ex vivo) in both animal and human studies. It is interesting to consider the mechanism that underlies the putative synergistic interaction between the l-arginine/NO pathway on the one hand and the arachidonic acid/prostaglandin pathway on the other. Such a possible mechanism was described by Salvemini and coworkers, who showed in an in vitro study that in endothelial cells, NO-mediated activation of COX leads to PGI2 release and cAMP elevation, contributing to the antithromboembolic effect of endogenously released NO. The exact molecular mechanisms by which NO activates the COX enzyme remain to be identified. Several possible mechanisms have been proposed by Salvemini and coworkers, most of them involving superoxide radicals. Another possible mechanism underlying synergism includes cGMP-mediated inhibition of cAMP-phosphodiesterase, thus reducing cAMP degradation, as well as activation of certain target proteins that can be phosphorylated by protein kinases dependent on both cGMP and cAMP. This has been demonstrated for a 46-kDa “vasodilator-stimulated phosphoprotein” in platelets. If these mechanisms are specific for arteriolar endothelium, these in vitro findings may provide an explanation for our observation that in arterioles, the antithromboembolic effect of endogenous NO alone is negligible but that it is pronounced in combination with endogenous prostaglandins, provided sufficient NO is produced to activate the arachidonic acid/prostaglandin pathway.

The effects of inhibition of the endogenous production of either NO or prostaglandins were similar to those observed in previous studies from our institute. In addition, our finding that in the L-NA group the embolization parameters were similar in arterioles and venules is in agreement with the observation in one of these studies. Apparently, L-NA superfusion abolishes the difference in embolization duration between arterioles and venules, which is larger in the arterioles under control conditions. It is unknown whether the insignificant role of NO alone in inhibiting thromboembolism in arterioles is due to low local NO synthesis by the arteriolar wall or to a limited bioavailability of NO in arterioles in vivo. By means of immunohistochemistry, we showed a similar expression of endothelial NO synthase in arterioles and venules, making a reduced capacity for NO synthesis in arterioles unlikely. A low bioavailability of NO in arterioles may be due to the scavenging effect of superoxides, which at least in rats are generated in higher quantities in mesenteric arterioles than in venules. In the present study, ASA was used for control and selective irreversible inhibition of the enzyme COX. However, an influence on other enzymes or proteins, such as kinases involved in the nuclear factor-κB pathway, cannot be excluded. This inflammatory pathway is activated by such stimuli as tumor...
necrosis factor-α. Because our experiments were conducted under near-physiological circumstances, it is unlikely that this pathway played a role in our study. The findings in the present study and those in an earlier study from our institute6 show that the involvement of both proaggregatory prostaglandins (eg, thromboxane A2) and antiaggregatory prostaglandins (eg, PGI2) in thromboembolic processes is more pronounced in arterioles than in venules. This can likely be explained by a functional difference between arteriolar and venular endothelium; damaged or stimulated endothelial cells seem to synthesize more antplatelet and proplatelet prostaglandins in arterioles than in venules.

It is unlikely that the route of administration of L-NA and ASA accounts for the difference in effect between arterioles and venules. In a previous study, we provided evidence that superfused L-NA enters the lumen of mesenteric arterioles and venules similarly,7 whereas the intravenous administration of ASA most likely results in similar plasma concentrations in arterioles and venules.6 Beside endothelial cells, blood platelets also constitutively express NO synthase and COX. It is unlikely that the release of NO and prostaglandins by platelets is different in arterioles and venules, because electron and light microscopy showed that the composition of the stationary thrombus (ie, mainly tightly packed platelets) is not different in the 2 types of microvessels.20 Moreover, the size of the stationary thrombus was similar in arterioles and venules. Therefore, we conclude that the different effects of L-NA and ASA on thromboembolism in arterioles and venules have to be accounted for by differences in vessel wall properties.

In conclusion, this is the first study to provide evidence of a synergistic effect of endogenous NO and prostaglandins in protecting against ongoing thromboembolism after vessel wall injury in arterioles but not in venules. Furthermore, this study indicates that under normal circumstances, endogenous NO and prostaglandins counteract proaggregatory effects of wall shear rate in arterioles. In venules, other, as-yet- unidentified antithrombogenic substances are likely to play a role.

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References


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