Abstract—Nitric oxide (NO) exerts important vasodilatory, antiplatelet, antioxidant, antiadhesive, and antiproliferative effects. Although endothelium derived NO has been shown to be of prime importance in cardio- and vasculoprotection, until recently little was known about the role of platelet-derived NO. New evidence suggests that NO synthesized by platelets regulates platelet functions, in particular suppressing platelet activation and intravascular thrombosis. Moreover, platelet NO biosynthesis may be decreased in patients with cardiovascular risk factors or with coronary heart disease, and this may contribute to arterial thrombotic disease in these patients. Here, we review the current state of knowledge as regards the role of platelet-derived NO, both in normal physiology and in cardiovascular disease states, and compare platelet NO signaling and regulation with that in endothelial cells. (Circ Res. 2007;101:654-662.)

Key Words: platelets ■ nitric oxide ■ cardiovascular physiology ■ cardiovascular disease

Background: Nitric Oxide in the Vasculature

Nitric oxide (NO) is formed from the amino acid L-arginine by a 2-step oxidation of L-arginine to L-citrulline catalyzed by the enzyme nitric oxide synthase (NOS).1 Of the 3 isoforms identified to date, NOS3 (endothelial-type NOS, NOS III) is expressed constitutively in endothelial cells and is the main source of vascular NO under physiological conditions.2–4 although small amounts of NOS2 (inducible NOS, NOS II) have also been reported to be expressed in the endothelium.5 Soluble guanylyl cyclase (sGC) is the second messenger enzyme for NO. After its synthesis in the endothelial cell, NO diffuses to the subjacent vascular smooth muscle cells, where it activates sGC (a heme-containing enzyme),6 thereby leading to the production of cyclic guanosine monophosphate (cGMP). By inducing vasodilation, endothelium-derived NO contributes to the maintenance of basal vascular tone and blood flow, and thus to the physiological regulation of blood pressure7,8; it also has important antiplatelet, antioxidant, antiadhesive, and antiproliferative properties.9–18 Other cells expressing NOS3 in the cardiovascular system include cardiac myocytes,9 red blood cells, megakaryocytes, and platelets.10,11 Here we review the physiological and pathophysiological role of platelet-derived NO, as well as the current state of knowledge as regards platelet NO signaling and regulation and how this compares with endothelial NO signaling and regulation.

The L-arginine-NO Pathway in Platelets

Platelets produce NO in smaller amounts than do endothelial cells.9,10 Both the NOS2 and NOS3 isoforms have been described in platelets,11 but NOS3 is predominant.12 In 1990, Radomski and colleagues demonstrated that, in platelets activated by collagen, L-arginine inhibits aggregation, stimulates sGC, and increases cGMP levels.9 Platelet NOS3 was shown to be Ca\textsuperscript{2+}/calmodulin-regulated and to require NADPH for its activity.9,13 in the same way as NOS3 in endothelial cells. The same workers further showed that other aggregating agents such as ADP and arachidonic acid could similarly induce an increase in platelet NOS3 activity; furthermore, L-arginine was found to inhibit platelet aggregation and N\textsuperscript{6}-monomethyl-L-arginine (L-NMMA), a non-specific NOS inhibitor, was shown to enhance aggregation and to inhibit cGMP production in response to these agents.10 They concluded that an increase in intracellular Ca\textsuperscript{2+} attributable to stimulation of platelets by aggregating agents activates platelet NOS3 and leads to NO production, and proposed that NO generated from platelets acts as a negative feedback mechanism regulating platelet activation.10 It appears therefore that, by contrast to the situation in endothelial cells where NO produced diffuses to adjacent cells to exert its action in a paracrine manner, platelet-derived NO exerts its effects within the same cell in which it is produced (that is to say, in an autocrine manner).

NOS3 mRNA and protein have both been detected in platelets, both in health and in cardiovascular disease states.12,14–16 Evidence that platelets produce NO both under resting conditions and after stimulation have come from studies using the conversion of oxyhemoglobin to methemoglobin, and the generation of nitrite and nitrate.17 Malinski and colleagues reported that activated platelets release approximately 10\textsuperscript{-11} mol NO/min/10\textsuperscript{8} platelets, as measured electrochemically.18 Similarly, Freedman and colleagues found similar levels of NO release by ADP-activated platelets, whereas platelets from NOS3-deficient mice produced no detectable levels of NO.19 Additionally, platelets have been found to have a measurable basal level of NOS activity,
as determined from the conversion of L-arginine to L-citrulline.12,16,20

**Regulation of Platelet NOS3 Activity and NO Production: Differences From Endothelial Cells**

NOS3 in platelets was initially considered similar to that found in endothelial cells, in terms of its dependence on intracellular Ca\(^{2+}\) for activation. Although platelet NOS3 was initially characterized as a Ca\(^{2+}\)-calmodulin sensitive enzyme,10 recent evidence suggests that activation of this enzyme more usually occurs independently of intracellular Ca\(^{2+}\). Although it is true that stimuli that increase intracellular Ca\(^{2+}\) such as ADP and thrombin activate platelet NOS3 and induce NO generation,10,21 many other stimuli can activate the enzyme with no detectable effect on intracellular Ca\(^{2+}\). β-Adrenoceptors (βAR), which are present on platelets and couple to adenyl cyclase (AC),22,23 can stimulate NOS3 activity and NO biosynthesis, by increasing cAMP (cyclic adenosine monophosphate) and protein kinase A (PKA) activity.20 This effect is mediated through the β\(_2\)-subtype of βAR and is not associated with any change in intraplatelet Ca\(^{2+}\). β-AR stimulation inhibits platelet adhesion to human umbilical vein endothelial cell monolayers, an effect abolished by NOS inhibition.20 The sole involvement of the AC-cAMP-PKA pathway in β\(_2\)-AR-mediated NOS3 activation in platelets contrasts with the situation reported in human umbilical vein endothelial cells, where β\(_2\)-AR-mediated NOS3 activation involves both PKA and protein kinase B (Akt).24,25

We have recently demonstrated that platelet NOS3 binds to the globular, but not to the filamentous, form of β-actin, and the affinity of this binding is substantially increased by heat shock protein 90 (Hsp90). Binding results in the formation of a ternary complex (NOS3/globular β-actin/Hsp90), localized to platelet caveolae, and thereby activation of NOS3. Thus the conformational state of β-actin has a major influence on NOS3 activity in platelets, and we found that this occurs independently of intracellular Ca\(^{2+}\). The formation of this ternary complex in turn gives rise to an increase in Hsp90 degradation, which acts as a negative feedback on NOS3 activation in platelets.26

Catecholamines and adenosine stimulate platelet NO production, effects that are prevented by NOS inhibition.27,28 This has been linked to the activation of PKA and subsequent phosphorylation of the Ser\(^{1177}\) residue in NOS3.29 Phosphorylation of the Ser\(^{1177}\) residue increases NOS3 catalytic activity by increasing electron flux at the reductase domain and by reducing calmodulin dissociation from activated NOS3 when Ca\(^{2+}\) levels are low. Insulin also increases NOS3 activity in platelets, in a manner which is Ca\(^{2+}\)-insensitive but is also dependent on phosphorylation on Ser\(^{1177}\).30 However, unlike catecholamines or adenosine, the effect of insulin appears to depend on activation of Akt, as it is inhibited by wortmannin, an inhibitor of phosphatidylinositol 3-kinase (PI3-K) which is the upstream activator of Akt.31,32 In contrast to its effect in endothelial cells, stimulation of platelet NOS3 activity by insulin leads to an increase in platelet cGMP of 2- to 4-fold, significantly higher compared with the increase in platelet cGMP induced by collagen; this in turn attenuates thrombin-induced platelet aggregation, an effect which is prevented by NOS inhibition.31,32 The stimulatory effect of insulin on platelet NOS3 is partially dependent also on AMP-dependent protein kinase activation, again through phosphorylation of NOS3 on Ser\(^{1177}\). It may also be partly dependent on Hsp90, as inhibition of the association of Hsp90 with NOS3 by geldanamycin attenuates the activation of the enzyme and cGMP production, and also diminishes the inhibitory effect of insulin on platelet aggregation.31 This contrasts with the situation in endothelial cells where, although insulin application induces phosphorylation of NOS3 by Akt, this is not associated with a measurable increase, in the short-term, in NO biosynthesis, or with relaxation of endothelium-intact arterial segments33,34; on the other hand, after 18 to 24 hours’ exposure, insulin does give rise to an increase both in NOS3 mRNA and in intracellular cGMP in endothelial cells.35

Recently, the signaling pathway through which collagen activates platelet NOS3 and induces cGMP production has been investigated. The effect of collagen is mediated through its receptor glycoprotein (GP) VI, because blockade of the other collagen receptor α\(_2\)β\(_1\) in platelets does not affect collagen-induced cGMP production. This pathway requires an increase in intracellular Ca\(^{2+}\) and activation both of the PI3K-Akt pathway and of protein kinase C (PKC).35 Platelet stimulation by collagen gives rise to secretion of ADP and thromboxane A\(_2\) (TxA\(_2\)), which in turn can also enhance platelet cGMP production.35 On the other hand, other studies have found no change in intraplatelet Ca\(^{2+}\) after stimulation with collagen, even though activation of the NO pathway occurs.36,37 The discrepancies found in these studies may be explained by methodological differences in intraplatelet Ca\(^{2+}\) measurement, and further studies are needed to clarify the role of Ca\(^{2+}\) in the modulation of collagen-mediated NO production by platelets.

Other stimuli that activate platelet NOS3 include shear stress38 and α-tocopherol.39 In a system where platelet adhesion and aggregation on a collagen type III surface was measured, it was found that shear stress reduced, and NOS3 inhibition enhanced, platelet coverage of the surface, independently of intracellular Ca\(^{2+}\).38 α-tocopherol, both in vitro and in vivo, increases platelet NO release during ADP-induced platelet aggregation, and this occurs partially through superoxide (O\(_2^•\)) scavenging but also by inhibition of PKC; the latter has been reported to mediate NOS3 phosphorylation on Thr\(^{495}\) which may have functional consequences as described below.39,40

Other endogenous factors that may be involved in the regulation of platelet NOS3 activity include von Willebrand factor (vWF) and glucose.41,42 vWF activates platelet NOS3 and increases intraplatelet cGMP, as well as inducing phosphorylation of vasodilator-stimulated phosphoprotein (VASP, a cGMP substrate). It does so by acting through its receptor GPIIb, and in a Ca\(^{2+}\)-sensitive manner which requires the participation of PI3-K and phospholipase C; it also involves the generation of ADP and TxA\(_2\), because vWF-induced cGMP production is blocked by apyrase and indomethacin.41 High glucose activates NOS3 in resting platelets by an osmotic mechanism that probably involves the β isoform of
PKC, activation of which causes an increase in intraplatelet Ca^{2+}.

Although the importance of phosphorylation of NOS3 in the regulation of its activity in platelets is well accepted, only 1 phosphorylation site, namely that on Ser^{1177}, is currently established as being of importance in this regard. This contrasts with NOS3 in endothelial cells, whose activity is known to be modulated by phosphorylation on a variety of residues. In endothelial cells, NOS3 can be phosphorylated on Ser^{1177} by the action of Akt,24,44,45 PKA,24,46 calmodulin-dependent kinase II,40 and AMP-activated protein kinase47; on Ser^{417} by Akt and PKA48; on Ser^{633} by PKA49; and on Thr^{495} by PKC.40 Phosphorylation of endothelial NOS3 on Ser^{1177}, Ser^{417}, and Ser^{633} activates the enzyme, whereas phosphorylation of Thr^{495} inhibits it. The role (if any) of phosphorylation of platelet NOS3 on Ser^{617}, Ser^{633}, or Thr^{495} in the modulation of its function remains to be established.

Additionally, in endothelial cells a variety of other proteins—Hsp90, NOS3-interacting protein (NOSIP), NOS3 traffic inducer (NOSTRIN), dynamin-2, and porin—appear to play an important role in the regulation of NOS3 activity. In endothelial cells, Hsp90 is found associated with NOS3 even in the resting state, but this association is enhanced on exposure to NOS3 agonists (including VEGF, histamine, and fluid shear stress) which in turn results in NOS3 activation. In platelets, Hsp90 has been shown to associate with NOS3 and to enhance its activity in response to insulin31 and after binding to the globular form of β-actin.26 In endothelial cells, NOSIP and NOSTRIN both inhibit,52,53 and dynamin-2 and porin both augment,54,55 NOS3 activity. However, at present nothing is known about the possible regulation of NOS3 in platelets by these proteins.

To date, little information is available on therapeutic interventions that may enhance platelet NO release. In a recent study, it was demonstrated that inhibition of the GPIIb/IIIa receptor, which is essential for fibrinogen binding and for a sustained platelet aggregation response, leads to an increase in platelet NO release and to attenuation of platelet O_2^- production on activation.56 In another study, aspirin was found to activate platelet NOS3 acutely, while at the same time inhibiting β2AR-mediated NOS3 activation.57 Further work is needed to explore other therapeutic options for enhancing platelet NO release, and to investigate whether such therapies may be clinically useful.

A summary of various stimuli which activate NOS3 in platelets, and the mechanisms by which they do so, is shown in Figure 1.

**The Physiological Role of Platelet-Derived NO**

At sites of vessel injury, platelets are rapidly activated. The first detectable event that occurs after platelet activation is shape change. Platelets lose their discoid shape and become spherical with spiky pseudopods and an irregular membrane. The change in shape increases their procoagulant activity.58 During these shape changes, myosin associates with the actin filaments so that platelet granules are contracted toward the center of the cell and their contents can be subsequently secreted.59 After shape change, platelets adhere to the exposed subendothelial matrix through the interaction of vWF with its receptor GPIb-IX-V, which is constitutively expressed on the platelet surface.60 Stable adhesion then follows through the binding of collagen with its platelet receptor α_5β_1,51,62 and of vWF with GPIIb-IIIa.63 The events that follow shape change and platelet adhesion include primary (reversible) aggregation and subsequently secondary (irreversible) aggregation, known also as platelet recruitment, after the release reaction. Essential for the primary aggregation of platelets is the binding of fibrinogen to its receptor, GPIIb-IIIa.64 Platelet activation results in a conformational change of GPIIb-IIIa which facilitates fibrinogen binding.65 Fibrinogen bound to its activated receptor cross-links adjacent platelets, leading to aggregation and thrombus formation. Platelet recruitment occurs mainly because of the release reaction in platelets. This includes the release of contents from the dense granules (such as ADP and Ca^{2+}) and α-granules (such as fibrinogen), as well as the de novo synthesis of prostaglandins and TxA_2. The release reaction results in an amplification of platelet responses, leading to irreversible aggregation and to stable thrombus formation.58
NO stimulates sGC in platelets, causing an increase in cGMP and hence activation of cGMP-dependent protein kinase (PKG). This in turn causes inhibition of platelet activation through various pathways. PKG promotes sarcoplasmic reticulum ATPase (SERCA)-dependent refilling of intraplatelet Ca\(^{2+}\) stores, thereby inhibiting influx of Ca\(^{2+}\) and other cations and decreasing intracellular Ca\(^{2+}\) levels. It also inhibits inositol-1,4,5-trisphosphate-stimulated Ca\(^{2+}\) release from the sarcoplasmic reticulum, which also contributes to decreasing cytosolic Ca\(^{2+}\). PKG also phosphorylates the TxA\(_2\) receptor, thereby inhibiting its function. In addition, 2 other mechanisms have been identified whereby cGMP prevents platelet activation. Firstly, cGMP indirectly increases intracellular cAMP through inhibition of phosphodiesterase type 3; cGMP and cAMP act synergistically to inhibit platelet aggregability. Secondly, it inhibits the activation of PI3-K, which in turn causes activation of GP IIb-IIIa fibrinogen receptors.

Apart from the cGMP-dependent pathways described above, there is evidence that NO can also regulate platelet function independently of cGMP. Several NO donors, such as S-nitrosocysteine, S-nitrosoglutathione, diethylamine diazeni-umidolate, S-nitrosohemoglobin, and inhaled NO, can inhibit platelet aggregation in a manner which is not affected by inhibition of sGC. NO has also been shown to inhibit ATP-dependent Ca\(^{2+}\) uptake into platelet membrane vesicles in a manner which cannot be attributed to cGMP, because cGMP itself only has a weak effect on this uptake even at high concentrations. Recently, it has also been shown that NO inhibits exocytosis of platelet granules (dense, lysosomal, and \(\alpha\)-granules) by S-nitrosylation of N-ethylmaleimide-sensitive factor (NSF), an effect that is not prevented by sGC inhibition. NSF may therefore be an important cGMP-independent mediator of NO action in platelets; further work is necessary to clarify other cGMP-independent pathways in platelets through which NO may exert its effects.

Early experiments by Radomski and colleagues suggested that platelet-derived NO importantly regulates primary platelet aggregation. In these experiments, using prostacyclin-washed platelets, L-arginine inhibited and L-NMMA enhanced platelet aggregation induced by agonists such as collagen, ADP, and arachidonic acid. Later experiments by other groups failed to reproduce these results and suggested that platelet NO may not substantially modulate primary platelet aggregation. In these studies, again with prostacyclin-washed platelets, L-arginine and L-NMMA had no detectable effect on platelet aggregation induced by collagen, thrombin, or ADP, and L-arginine did not potentiate the inhibitory effect of glyceryl trinitrate on platelet aggregation. Furthermore, after inhibition of platelet NOS by L-NMMA, platelet aggregation induced by ADP was found not to be altered, and the inhibitory effect of the \(\beta\)AR agonist isoproterenol on platelet aggregation was not affected. The optical method for measurement of platelet aggregation, which has been widely used in these studies, has a number of limitations. For example, it detects only the formation of macro-aggregates, but not micro-aggregates. It is also known that centrifugation may in itself cause platelet activation and desensitization to aggregating agents. Additionally, the removal of red blood cells, to obtain platelet-rich plasma, causes an increase in pH and deterioration of platelet function over time. This method may therefore be too insensitive to detect early or subtle changes in platelet function. It is therefore possible that the discrepancies observed in the above studies may be, in part, attributed to subtle uncontrolled differences in experimental conditions.

On the other hand, it has been shown that, although NOS inhibition appears to have no consistent effect on thrombin-induced platelet aggregation, it reduces the inhibitory effect of insulin on platelet aggregation. Similarly, the inhibitory effect of adenosine on platelet aggregation can be partially prevented by NOS inhibition. This suggests that, while platelet-derived NO does not necessarily and consistently inhibit platelet aggregation in response to proaggregants, it may enhance the antiplatelet effects of antiaggregatory mediators. Recent evidence also indicates that whereas many endogenous substances can inhibit platelet aggregation, platelet-derived NO appears to be unique in that it contributes importantly to the process of platelet disaggregation.

Although the role of platelet-derived NO in regulating primary platelet aggregation remains controversial, its importance on regulating other aspects of platelet activation is more established. Platelet-derived NO inhibits recruitment of platelets to the growing thrombus. This process is initiated by activated platelets at the site of vascular injury by secretion of ADP, serotonin, and TXA\(_2\), and further promotes thrombin deposition and thrombus formation on the platelet surface. In vitro, platelet-derived NO inhibits heterotypic aggregation between leukocytes and platelets, and in particular between monocytes and platelets, an early and robust marker of platelet activation implicated in the mechanism of atherogenesis and thrombosis. Inhibition of platelet NOS3 increases the expression of P-selectin on the platelet surface after stimulation with ADP, which is essential for the interaction between leukocytes and platelets. Inhibition of platelet NOS3 enhances the formation of monocyte-platelet aggregates, whereas exogenous NO decreases the extent of leukocyte-platelet complex formation.

It has also been shown that \(\beta\)AR-stimulated platelet NO production inhibits platelet adhesion to endothelial cells, although a possible contribution of endothelial-derived NO in these experiments cannot be excluded. Platelet-derived NO modulates the rate of thrombus growth on a collagen III surface, through altering platelet adhesion to this surface, and is sensitive to insulin, isoproterenol, or shear stress. Collectively, these data support a physiological role of platelet-derived NO in the modulation of platelet function and hence thrombus formation.

On the other hand, male NOS3 knockout mice, although having a reduced lifespan, die not from thrombotic complications but from cardiac failure, whereas female NOS3 knockout mice have a normal lifespan. Further, although NOS3-deficient mice have increased blood pressure and decreased heart rate, and aortic rings from these animals exhibit impaired endothelial-dependent response to acetylcholine, their platelets do not express more P-selectin on their surface and do not produce more thromboxane B\(_2\),—both markers of platelet activation. Similarly, no difference is
seen in the expression of GPIIb or in ADP-induced aggregation between NOS3-deficient and wild-type mice.93 However, when thrombocytopenic NOS3-deficient mice are transfused with platelets from NOS3-deficient mice, they exhibit decreased bleeding times compared with when they are transfused with platelets from wild-type mice; this effect has been attributed to enhanced platelet recruitment in the NOS3-deficient mice, as estimated by serotonin release from activated platelets, attributable to a reduction in platelet NO release.19

The picture is complicated further by some evidence which indicates that, whereas at higher concentrations NO inhibits platelet function, at lower concentrations it may paradoxically activate platelets, and this activation appears to be cGMP-mediated. PKG knockout mice exhibit impaired platelet activation in response to vWF and to low doses of thrombin, which occurs through impaired GPIb-IX-induced activation of GPIIb-IIIa.94 Similarly, in human platelets, these same workers found that aggregation induced by vWF or low-dose thrombin was prevented by PKG inhibition but was enhanced by cGMP; and furthermore, increasing intraplatelet cGMP with sildenafil promoted vWF- or thrombin-induced platelet aggregation.94 They concluded that cGMP-stimulated platelet responses are biphasic, consisting of an initial transient stimulatory response that promotes platelet aggregation and a subsequent inhibitory response that limits the size of thrombi. In another study, platelets from PKG I knockout mice and human platelets treated with a PKG inhibitor showed diminished aggregation-dependent exocytosis as well as a diminished secondary wave of platelet aggregation induced by a TxA2 analog and by thrombin receptor-activating peptides; low dose collagen-induced platelet exocytosis and aggregation were also reduced by PKG inhibition.95 These data suggest a potential for low concentrations of NO to activate rather than inhibit platelets, but whether this is truly so remains the subject of much controversy.

Figure 2 summarizes the physiological functions, both established and putative, of platelet-derived NO.

The Role of Platelet-Derived NO in Cardiovascular Disease

Numerous diseases affecting the cardiovascular system are characterized by impairment of endothelium-dependent vasodilation and NO release. Diminished production of endothelial NO, as determined from endothelium-dependent vasodilatory responses, has been reported in coronary heart disease,96,97 as well as in patients with coronary risk factors such as smoking,98 ageing,99 and hypercholesterolemia.100,101 Regarding diabetes mellitus and hypertension, results have been more controversial as many,102–105 but not all,106,107 studies have shown an impairment in endothelium-derived NO release. These conflicting findings are mainly attributed to the heterogeneous nature of patients with these diseases, and to factors related to disease duration and the presence of complications.

Diminished endothelial production of NO may coexist with a parallel decrease in platelet NO release, the combination thereby aggravating the increased thrombotic tendency present in these conditions. The role of platelet-derived NO in the context of cardiovascular diseases and cardiovascular risk factors has been investigated. Freedman and colleagues studied the clinical relevance of platelet-derived NO in patients with coronary heart disease, and specifically stable angina and acute coronary syndrome. Platelets from patients with unstable angina or acute myocardial infarction were found to produce significantly less NO in response to stimulation, as measured with an NO-selective microelectrode, than did those with stable angina pectoris.108 Furthermore, platelet-derived NO production was found to be an independent predictor of the incidence of acute coronary syndromes. In a more recent study, an inverse correlation was found between platelet NO production and the number of coronary risk factors. A multiple stepwise regression analysis model indicated that age, smoking and mean arterial pressure were the main factors which predicted platelet NO biosynthesis.109 Smoking has been reported to impair platelet NO generation. Platelets from long-term smokers release less NO during
Oxidized LDL-cholesterol stimulates platelet activation through a reduction in L-arginine transport into platelets and in platelet NOS3 expression.113 Consistent with this, a recent study in rats showed that statin treatment enhances platelet NO release and upregulates platelet NOS3 mRNA expression.112

In the context of diabetes, basal platelet NOS activity has been found to be decreased in both type I and type II diabetes mellitus as compared with healthy individuals.113 Furthermore, β2AR-mediated NO generation and cGMP production has been found to be impaired in platelets from patients with type II diabetes, and platelet NO-attributable cGMP levels correlate inversely with both HbA1c and fasting blood glucose levels.15 These findings suggest that type II diabetes may be associated with suppression of platelet NOS3 activation, to an extent dependent on the degree of glycemic control, and this may contribute to the increased platelet activation seen in this condition.

Considerable evidence suggests that platelet NO signaling is impaired in essential hypertension. Camilletti and colleagues found that platelet NO production, as estimated from nitrite levels, is reduced in hypertensive patients.114 Platelet L-arginine transport has also been reported to be reduced in hypertensives, attributable to downregulation of the membrane y+L transport system.115,116 Asymmetric dimethylarginine plasma levels are greater in hypertensive patients compared with normotensive controls,117 and this may give rise to enhanced inhibition of platelet NOS3 in hypertensives. Indeed, patients with recently diagnosed untreated mild essential hypertension have been found to exhibit impairment in stimulated platelet NOS3 activity; in this study, although albuterol and collagen both increased platelet NOS3 activity in normotensive subjects, they failed to generate such an increase in hypertensives.118 As these 2 agonists stimulate NOS3 through different pathways, it is likely that a generalized defect exists in the ability of platelet NOS3 to undergo stimulation in the context of hypertension.

In accordance with the previously reported decrease in endothelium-derived NO biosynthesis with age,99 platelet NOS3 activation has been found to be impaired in older (>45 years old) compared with younger (<30 years old) subjects; and, in accordance with this, intraplatelet cGMP levels are reduced in older compared with younger subjects.37

Collectively these studies demonstrate that, as for endothelium-derived NO, platelet NO generation is reduced in acute coronary heart disease and in patients with a variety of cardiovascular risk factors. Given the important effects described above of platelet-derived NO on platelet physiology, a decrease in platelet NO may play a role in the pathogenesis of the thrombotic complications of cardiovascular diseases and contribute to the increased platelet activation observed in these conditions. Although numerous interventions can improve endothelial NO availability and hence endothelium-dependent vasodilatation,119–122 no data yet exist as to whether interventions that can improve platelet NO availability or signaling can give rise to beneficial effects on platelet function, which may be of therapeutic benefit in terms of prevention of arterial thrombotic disease. Indeed, given that interventions that measurably improve endothelial NO biosynthesis (such as estrogen) have not been shown to positively influence cardiovascular outcome, it will be especially important to determine whether such is the case also for therapies that increase platelet NO generation.

Conclusions

Although NOS3 signaling and regulation shares many similarities between endothelial cells and platelets, important differences also exist; and because NO biosynthesis has been studied to a much greater degree in endothelial cells than in platelets, much still remains to be clarified about NOS3 signaling and regulation in platelets. Despite the fact that platelets produce much less NO than do endothelial cells, platelet-derived NO has important effects on platelet function and this is likely to be important in situations where platelets are in close proximity to each other, and specifically in the region of the growing thrombus. Platelet-derived NO has a potent modulatory effect on platelet recruitment, adhesion, and heterotypic aggregation to leukocytes. Its generation is impaired in cardiovascular diseases and in the presence of coronary risk factors and may, in combination with the well-documented decrease in endothelium-derived NO in these conditions, contribute to the occurrence of thrombotic complications.

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None.

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Eugenia Gkaliagkousi, James Ritter and Albert Ferro

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