Activation of Platelet Function Through G Protein–Coupled Receptors

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Abstract—Because of their ability to become rapidly activated at places of vascular injury, platelets are important players in primary hemostasis as well as in arterial thrombosis. In addition, they are also involved in chronic pathological processes including the atherosclerotic remodeling of the vascular system. Although primary adhesion of platelets to the vessel wall is largely independent of G protein–mediated signaling, the subsequent recruitment of additional platelets into a growing platelet thrombus requires mediators such as ADP, thromboxane A₂, or thrombin, which act through G protein–coupled receptors. Platelet activation via G protein–coupled receptors involves 3 major G protein–mediated signaling pathways that are initiated by the activation of the G proteins G₉, G₁₃, and G₁. This review summarizes recent progress in understanding the mechanisms underlying platelet activation and thrombus extension via G protein–mediated signaling pathways. (Circ Res. 2006;99:1293-1304.)

Key Words: platelet activation ■ heterotrimeric G proteins ■ GPCRs ■ thrombosis

Platelet adhesion and activation at sites of vascular wall injury is initiated by a multistep process involving the interaction of platelets with the subendothelial extracellular matrix which contains adhesive macromolecules including collagen and von Willebrand factor (vWF).¹,² The initial interaction of platelets with the extracellular matrix under conditions of high shear rates and the subsequent strengthening of this interaction involves the platelet vWF receptor GP Ib/V/IX and the collagen receptor GPVI³–⁴ (which will be covered in a forthcoming review in this series). GPVI is unable to mediate adhesion but, via activation of the FcRγ chain, induces intracellular signaling processes which promote the inside-out activation of integrins such as α₃β₁ (GPIIIb/IIIa) or α₂β₁ (GPIa/IIa).⁴,⁵ The interaction of activated integrins with the extracellular matrix then mediates the firm adhesion of platelets to the injured vessel wall, resulting in the formation of a platelet monolayer.

During the next stage of platelet activation, a platelet plug forms through the recruitment of additional platelets from the circulation and their integrin α₃β₁-mediated aggregation (see Figure 1). The recruitment of additional platelets is mediated by a variety of locally accumulating mediators that are produced or released once platelet adhesion has been initiated and some level of platelet activation through platelet adhesion receptors has occurred. These mediators include ADP/ATP and thromboxane A₂ (TxA₂), which are secreted or released from activated platelets and thrombin, which is produced on the surface of activated platelets. These diffusible mediators have in common that they act via G protein–coupled receptors (GPCRs). Through the activation of G protein–mediated
signaling pathways, they can further increase their own formation and release, thus acting as positive-feedback mediators that amplify the initial signals to ensure the rapid activation and recruitment of platelets into a growing thrombus (Figure 1).

Thus, G proteins are centrally involved in the second phase of platelet-dependent thrombus formation. Pharmacological studies as well as work based on the analysis of mice lacking individual components of G protein–mediated signaling pathways have identified GPCRs, G proteins, and effector pathways regulating platelet activation (see Tables 1 and 2). This review describes the major GPCRs involved in platelet activation and summarizes recent data on the role of individual G proteins and their downstream effectors in the regulation of platelet functions such as integrin-mediated aggregation, degranulation, or platelet-shape change. Finally, the review discusses whether G protein–mediated signaling pathways could serve as targets for the development of new antiplatelet strategies.

**Platelet Activators Working Through G Protein–Coupled Receptors**

GPCRs constitute the largest family of proteins in the human genome.¹⁻³ They can be activated by a chemically very diverse group of ligands including amines, lipids, peptides, ions, nucleotides, or proteases.⁴ Because of their ability to specifically interact with various functionally different heterotrimeric guanine nucleotide-binding proteins (G proteins), agonist-activated GPCRs can induce different signaling pathways to change cellular functions.⁵⁻⁶ The large versatility of the G protein–mediated signaling system may explain why it is the primary mediator of the second phase of platelet activation during thrombosis and hemostasis, which requires the coordinated and fast action of a variety of diffusible mediators to activate platelets and to recruit them into the growing thrombus.

**Adenosine Diphosphate**

ADP is stored at high concentrations in dense granules of platelets and is released on platelet activation. Released ADP strongly activates platelets in an autocrine and paracrine fashion. It can also be released from damaged cells at places of vascular injury. Platelet activation by ADP is mediated by 2 G protein-coupled receptors, P2Y₁ and P2Y₁₂.¹²⁻¹³ Whereas P2Y₁ couples to G₁₂,¹⁴⁻¹⁵ P2Y₁₂ is coupled to G₁₅-type G proteins, in particular to G₁₅.¹⁶⁻¹⁷ Studies using receptor agonists had suggested that activation of both receptors is required for a full response of platelets to ADP.¹⁸⁻²⁰ Platelets from mice lacking P2Y₁ do not undergo shape change in response to ADP, and ADP-induced aggregation is severely impaired. In addition, mice lacking P2Y₁₂ have mildly increased bleeding times and a relative resistance to ADP-induced thromboembolism.²¹⁻²² Absence of P2Y₁₂ in humans results in a mild form of hemorrhagia,²³⁻²⁴ a phenotype not observed in mice lacking P2Y₁₂.²⁵⁻²⁶ Platelets from mice deficient in P2Y₁₂ have a normal shape change but an impaired aggregation in response to ADP.²⁵⁻²⁶ In addition, these animals have severely prolonged bleeding times and form smaller and unstable thrombi.²⁷ Both P2Y₁ and P2Y₁₂ are also involved in ADP-induced platelet procoagulant activity.²⁸ Interestingly, platelet responses to thrombin and TxA₂ at low and intermediate concentrations are reduced in the absence of ADP receptors.²¹⁻²³,²⁵,²⁶ underlining the important role of ADP as a positive-feedback mediator required for sustained platelet activation. The P2Y₁₂ receptor is irreversibly inhibited by thienopyridines such as clopidogrel, which is currently used for the secondary prevention of cardiovascular events.²⁹⁻³⁰

**Thrombin**

Thrombin is the main effector protease of the coagulation system and is among the most effective activators of platelets. Thrombin formation is initiated by the exposure of tissue factor to plasma coagulation factors after disruption of the vascular endothelium. Thrombin formation takes place on cellular surfaces including that of activated platelets.³¹ The local production of thrombin on the platelet surface represents an important mechanism by which activated platelets stimulate coagulatory processes. In addition, it may facilitate activation of platelets by thrombin, which is rapidly inacti-
vated after its formation. Activation of platelets by thrombin is mediated by protease-activated receptors (PARs), which couple to Gq, G12/G13, and, in some cases, also to the Gi family of heterotrimeric G proteins. Of the four protease-activated receptors, PAR1 and PAR4 are present on human platelets, whereas mouse platelets express PAR3 and PAR4. Studies using PAR1 antagonists or antibodies blocking PAR1 or PAR4 activation have indicated that PAR1 mediates human platelet activation at low thrombin concentrations, whereas PAR4 contributes to thrombin-induced platelet activation only at high thrombin concentrations. The higher potency of thrombin toward PAR1 activation is most likely attributable to the presence of a hirudin-like sequence close to the C-terminal thrombin cleavage site, which facilitates binding of thrombin and is absent in PAR4. The presence of PAR3 and PAR4 in mouse platelets suggested that they function analogous to PAR1 and PAR4 in human platelets. This was initially suggested by studies using platelets from PAR3-deficient mice, which did not respond any more to low and intermediate concentrations of thrombin but could be activated by high concentrations via PAR4. However, studies with heterologously expressed receptors revealed that mouse PAR3, which contains a hirudin-like sequence close to the thrombin cleavage site, did not mediate thrombin-induced transmembrane signaling unless PAR4 was present. This suggests that mouse PAR3 functions as a coreceptor for mouse PAR4 and facilitates cleavage and activation of PAR4 at low thrombin concentrations. Consistent with this, platelets from PAR4-deficient mice are unresponsive to thrombin and protected against thrombosis. Thus, although thrombin activates human platelets by cleaving and activating PAR1 and PAR4 at low and high concentrations, respectively, thrombin-induced mouse platelet activation is completely dependent on PAR4-mediated signaling and requires PAR3 only to facilitate cleavage of PAR4 at low thrombin concentrations. It is not known whether mouse PAR3 and PAR4 form stable heterodimers in which PAR3 mediates activation and PAR4 signaling, or whether binding of thrombin to PAR3 simply facilitates cleavage of PAR4.

**Thromboxane A2**

Like ADP, TxA2 functions as a positive-feedback mediator during platelet activation. It is produced from arachidonic acid through conversion by cyclooxygenase-1, the target of low-dose aspirin, and thromboxane synthase. The action of TxA2 is locally restricted because of its short half-life. The TxA2 receptor (TP), which is also activated by the prostanoid endoperoxides PGH2 and PGH3, couples to Gq and G12/G13. The role of TP as the platelet TxA2 receptor has been demonstrated in studies using platelets from TP-deficient mice, which become unresponsive to TxA2. TP-deficient mice have prolonged bleeding times and are unable to form stable thrombi. A reduced activation of TP-deficient platelets has been suggested to contribute to a reduced injury-induced vascular proliferation as well as to a reduced progression of atherosclerosis observed in mice lacking TP.

**Other Platelet Stimuli**

Several other stimuli have been identified that act through GPCRs. However, in contrast to ADP, thrombin, and TxA2, they are only weak activators of platelets and appear to serve primarily as potentiators of platelet responses to other stimuli.

For instance, epinephrine alone is not able to activate platelets but potentiates the effect of other stimuli by acting through the α2A-adrenergic receptor. Interestingly, the α2A receptor in platelets preferentially couples to the Gq-type G protein Gi. In mice lacking the α2A-adrenergic receptor, the potentiating effect of epinephrine on platelet activation was absent, bleeding time was moderately prolonged, and the formation of stable thrombi was impaired.

Prostaglandin E2 can potentiate platelet activation via the EP3 receptor. The EP3 receptor form expressed on platelets is believed to be coupled to Gq-type G proteins. In mice lacking EP3, bleeding times are increased and the potentiating effects of prostaglandin E2 are abrogated.

Serotonin is taken up by platelets, stored in dense granules, and released on platelet activation. Part of its action serves as a positive-feedback mechanism by activating 5-hydroxytryptamine 2A receptors on platelets, which are Gq-coupled.

Various chemokines such as platelet factor 4 (PF4/CXCL4), RANTES (CCL5), or CXCL5 are released from activated platelets. During recent years, it has been demonstrated that platelets express chemokine receptors such as CXCR4, the receptor of stromal cell-derived factors 1α (SDF-1/CXCL12), or CCR4, which is activated by macrophage-derived chemokine (MDC/CCL22) and by TARC (CCL17). Also, the chemokine receptors CCR1, CCR2, and CX3CR1, which are activated by RANTES (CCL5), MCP-1 (CCL2), and fractalkine (CX3CL1), respectively, have been shown to be expressed by platelets.

Chemokine receptors are coupled to Gq-type G proteins and mediate a rather weak activation of platelets. However, they are able to potentiate the effects of other platelet stimuli. There is increasing evidence that the secretion and presentation of chemokines by platelets, as well as the activation of platelets by chemokines, play an important role in the development of atherosclerotic vascular disease.

Lyosphospholipids such as lyosphosphatidic acid (LPA) can activate platelets. There is evidence that platelets express LPA1, LPA2, and LPA3 receptors, and related lyosphospholipid species are found in mildly oxidized low-density lipoprotein, as well as in advanced atherosclerotic lesions, and may contribute to platelet activation at various stages of atherosclerosis.

**Inhibitors of Platelets Acting Through GPCRs**

The major endothelium-derived inhibitors of platelet activation are nitric oxide (NO) and prostacyclin (PGI2), which raise the levels of the cyclic nucleotides cGMP and cAMP. Although NO directly activates guanylyl cyclase, PGI2 acts through a Gs-coupled receptor, the IP receptor, to stimulate adenylyl cyclase. Recent evidence suggests that PGI2-dependent platelet inhibition may play an important role in the protective effect of prostacyclin. In IP receptor-deficient mice, injury-induced vascular proliferation and platelet acti-
vation was enhanced. In mice lacking both IP and TP receptors, this augmented response was abolished, suggesting that PGI2 and TxA2, acting through their respective receptors, antagonistically regulate vascular and platelet functions.47 This complex functional antagonism may also explain the adverse cardiovascular effects associated with selective cycooxygenase-2 inhibitors, which inhibit the formation of PGI2 but not of TxA2.65

Adenosine released during cell damage or by conversion of ADP by the endothelial ectonucleotidase CD39 inhibits platelet function by activating the Gs-coupled A2A receptor.66

G Protein–Mediated Signaling Pathways Mediating Platelet Activation

The main diffusible platelet stimuli ADP, TxA2, and thrombin recruit platelets into a growing thrombus by activating multiple G protein–mediated signaling pathways to induce platelet-shape change, degranulation, and integrin $\alpha_{IIb}\beta_{3}$-mediated aggregation. They use distinct mechanisms to induce full platelet activation. ADP activates $G_{q}$ and $G_{i}$ through its receptors P2Y1 and P2Y12, whereas TxA2 and thrombin activate mainly $G_{q}$ and $G_{12}/G_{13}$ via the TxA2 receptor or the protease-activated receptors PAR1/PAR4 or PAR3/PAR4. Because all mediators can in turn increase the formation and release of thrombin, TxA2, and ADP, their effects are amplified and eventually all major G protein–mediated signaling pathways are activated. These positive-feedback mechanisms have obscured the analysis of the roles individual G protein–mediated signaling pathways play in platelet activation. Platelets lacking the $G_{i}$ subunits of individual G-protein subtypes have been used to study the function of the main G protein–mediated signaling pathways in platelet activation independently of the mediators and their respective receptors involved. In the course of these studies the G proteins $G_{q}$, $G_{13}$, and $G_{i2}$ have been demonstrated to play important roles in platelet activation (see Table 2).

The $G_{i}/G_{11}$ family of G proteins couples receptors to $\beta$ isoforms of phospholipase C (PLC), of which, especially the $\beta_3$ and the $\beta_1$ isoforms, are present in platelets. Activation of PLC results in the formation of IP3 and diacyl glycerol leading to an elevation of free cytoplasmic $[Ca^{2+}]_e$ and activation of protein kinase C (PKC), respectively. Although most cells in the mammalian organism express both $G_{i}$ and $G_{11}$, platelets are an exception in that they only contain

<table>
<thead>
<tr>
<th>Table 1. Main Platelet Stimuli Acting via GPCRs and Phenotypes Observed in Receptor-Deficient Mice</th>
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<tbody>
<tr>
<td><strong>Agonist and Receptor</strong></td>
</tr>
<tr>
<td>ADP</td>
</tr>
<tr>
<td>P2Y$_{12}$</td>
</tr>
<tr>
<td>Thrombin</td>
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<td></td>
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<td></td>
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<td>TxA2</td>
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<td></td>
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<tr>
<td>Serotonin</td>
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</table>

*The major Gq-type G protein activated via P2Y$_{12}$ is $G_{i2}$. †The murine PAR3 receptor does not itself mediate transmembrane signalling but is believed to function as a cofactor for cleavage and activation of PAR4. ‡It is not clear whether these defects are attributable to loss of TP on platelets or other vascular cells. NA indicates not analyzed.

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G-proteins G13, Gq, and Gi to the induction of platelet-shape change. So far, no physiological significance for the lack of G13 has been reported. G13, a member of the G12/G13 family, has been shown to regulate several signaling pathways of which the Rho/Rho-kinase–mediated pathway is the best established. Activated G13 binds and activates a subgroup of Rho-specific guanine nucleotide exchange factors,69,70 G12, the main member of the Gi family expressed on platelets couples receptors in an inhibitory fashion to adenylyl cyclase. In addition, Gq-type G proteins are a major source for βγ complexes, which are released on G-protein activation and can regulate a variety of channels or enzymes including adenylyl cyclases or phosphatidylinositol 3-kinases (PI3Ks).71 The latter enzyme produces phosphatidylinositol-3,4,5-trisphosphate, which activates a variety of downstream effectors including the serine/threonine kinase Akt/protein kinase B (PKB).72,73

**Platelet-Shape Change**

A shape change is the initial response of platelets to activators such as thrombin, ADP, or TxA2. It is an extremely rapid process based on the reorganization of the cytoskeleton.74,75 During platelet-shape change, new actin filaments are formed, leading to the formation of a submembranous actin filament network and the extension of filopodia. In addition, actomyosin-based contractile processes are stimulated, resulting in the centralization of dense and α granules. Finally, the circumferential microtubule coil depolymerizes, which allows the platelet to change from a discoid to a spherical shape. The platelet-shape change, which is induced by agonist concentrations lower than those required for degranulation and aggregation, is believed to be a prerequisite for efficient secretion of granule contents and to greatly facilitate adhesion of platelets to each other and to components of the extracellular matrix.

Platelet agonists can induce shape change under conditions in which they do not stimulate PLC or induce an increase in [Ca2+]i, suggesting that an elevation of [Ca2+]i alone is not sufficient to induce platelet-shape change.76–79 Consistent with that, thrombin and TxA2 can still induce platelet-shape change in the absence of Gq-mediated PLC activation.68 This suggests that other G proteins activated by thrombin and TxA2 receptors such as G12 and G13 are involved in the induction of platelet-shape change.34 In platelets lacking Gα13

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**Table 2. Platelet Phenotypes of Mice Lacking G Protein α Subunits**

<table>
<thead>
<tr>
<th>G Protein α Subunit</th>
<th>Effector</th>
<th>Platelet Phenotype In Vitro</th>
<th>Platelet Phenotype In Vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gα2</td>
<td>AC ↓; PIP3Kα/β ↑</td>
<td>Reduced aggregation and inhibition of cAMP formation in response to ADP and thrombin; normal shape change</td>
<td>NA</td>
<td>17,108</td>
</tr>
<tr>
<td>Gα3</td>
<td>AC ↓; PIP3Kα/β ↑</td>
<td>No apparent defect</td>
<td>NA</td>
<td>123</td>
</tr>
<tr>
<td>Gα2</td>
<td>AC ↓</td>
<td>Reduced aggregation and inhibition of cAMP formation in response to epinephrine; normal shape change</td>
<td>No overt bleeding defect in 1 study; increased bleeding time in the other; protected against thromboembolism induced by collagen/epinephrine</td>
<td>109,49</td>
</tr>
<tr>
<td>Gα4</td>
<td>PLC-β 1</td>
<td>Absence of IP3 production; Ca2+ transients; aggregation and secretion in response to TxA2; ADP and thrombin; normal shape change and RhoA activation in response to thrombin and TxA2 but not to ADP</td>
<td>Increased bleeding time; protected against collagen/epinephrine-induced thromboembolism</td>
<td>34,98,68</td>
</tr>
<tr>
<td>Gα12</td>
<td>RhoGEF ↑</td>
<td>No apparent defect</td>
<td>No apparent defect</td>
<td>80</td>
</tr>
<tr>
<td>Gα13</td>
<td>RhoGEF ↑</td>
<td>Reduced aggregation and secretion in response to TxA2 and thrombin; impaired shape change and RhoA activation</td>
<td>Increased bleeding time; protected against arterial thrombosis</td>
<td>80</td>
</tr>
<tr>
<td>Gα4+Gα13</td>
<td>See above</td>
<td>Unresponsive to thrombin, TxA2, and ADP</td>
<td>NA</td>
<td>81</td>
</tr>
</tbody>
</table>

*Activation of PIP3 is mediated by Gβγ. RhoGEF indicates Rho-guanine nucleotide exchange factor. AC indicates adenylyl cyclase.
but not Ge13, low and intermediate concentrations of thrombin and TxA2 were not able to induce a shape change. At high agonist concentrations, TxA2 and thrombin activated platelet-shape change also in the absence of Go13. However, in the absence of both Go13 and Goq, platelet-shape change could not be induced even at maximal agonist concentrations. This clearly indicates that platelet-shape change can be induced through Go as well as through G13. Stimuli that are able to activate both G proteins via their respective receptors such as TxA2 and thrombin preferentially use G13 to induce platelet-shape change. Stimuli such as ADP, which activates G2-mediated signaling pathways but not signaling via G13, induce platelet-shape change solely via Go. Activation of Go does not appear to be required for the induction of platelet-shape change (see Figure 2).

The regulation of myosin light chain (MLC) phosphorylation has been suggested to be involved in the induction of platelet-shape change. MSC phosphorylation can be controlled through a Ca2+/calmodulin-dependent regulation of MLC kinase and through a Rho/Rho-kinase–mediated regulation of myosin phosphatase. In Goq-deficient platelets the agonist-induced platelet-shape change could be blocked by inactivation of Rho and inhibition of Rho-kinase, and MLC phosphorylation and shape change in response to TxA2 and low concentrations of thrombin were strongly dependent on the Rho/Rho-kinase pathway in wild-type platelets. The central role of the Rho/Rho-kinase–mediated regulation of MLC phosphorylation and platelet-shape change is supported by the strong correlation between the ability of stimuli to induce platelet-shape change and their ability to stimulate RhoA activation and MLC phosphorylation. In platelets lacking Goq, TxA2 and thrombin were still able to induce shape change, RhoA activation, and MLC phosphorylation. High concentrations of TxA2 and thrombin as well as ADP are able to induce platelet-shape change as well as RhoA activation and MLC phosphorylation also in the absence of Goq. However, in the absence of both Goq and G13, none of the stimuli can induce platelet-shape change or activation of RhoA or MLC phosphorylation. This indicates that activation of the Rho/Rho-kinase–mediated signaling pathway is required for agonist-induced MLC phosphorylation in platelets. When the G13-mediated signaling pathway resulting in Rho/Rho-kinase activation is blocked, Gq-mediated MLC phosphorylation can be induced only under sufficiently high agonist concentrations which result in RhoA activation through Gq. Gq has been shown to be able to mediate RhoA activation in a PLC-independent manner via Rho guanine nucleotide exchange factors. Stimulation of Rho/Rho-kinase and MLC phosphorylation increases actomyosin contractility, and activation of Rho-kinase has been suggested to be required for the dynamic regulation of microtubule coils during platelet-shape change.

In addition to MLC phosphorylation, several other signaling processes may be involved in agonist-induced platelet-shape change. This includes tyrosine kinases such as pp60c-src or pp72syk. Actin assembly during platelet-shape change has also been shown to be controlled by polyphosphoinositides such as phosphatidylinositol 4,5-bisphosphate. Formation of phosphatidylinositol 4,5-bisphosphate by phosphatidylinositol 4-phosphate 5-kinase can be regulated by the small GTP-binding protein Rac, as well as by RhoA. Rac becomes activated in platelets on receptor activation and can also induce actin polymerization through the activation of the Arp2/3 complex mediated by WAVE proteins. However, in Goq-deficient platelets, activation of Rac by various agonists is abrogated while they are still able to induce platelet-shape change, indicating that Rac activation is not required for agonist-induced platelet-shape change. Under these conditions, actin assembly and polymerization may be induced through other RhoA-dependent pathways such as the activation of 4-phosphate 5-kinase or of formins, which promote linear elongation of actin filaments.

Platelet Aggregation

The accumulation of platelets into a hemostatic thrombus is based on the formation of multiple platelet/platelet interactions (platelet aggregation). Platelet aggregation is mediated by integrin β3, which binds various extracellular macro-molecular ligands including fibrinogen and vWF. The dimeric structure of fibrinogen and the multimeric structure of vWF allow these ligands to crossbridge platelets and to generate a platelet aggregate. In unactivated, resting platelets, αqβ is in an “off” state, in which ligand binding affinities are low and no signaling occurs. Platelet activation induces intracellular signaling processes that influence the cytoplasmic part of αmβ3, rapidly converting αmβ3 into an active conformation (inside-out signaling), which results in fibrinogen/vWF-mediated platelet aggregation.

Although the exact signaling mechanisms that link receptors of platelet activators to the cytoplasmic domains of αmβ3 are incompletely understood, the role of different G-protein subfamilies and the role of some of their immediate effector pathways have been described during recent years. There is good evidence that activation of PLCβ through Goq, which results in the formation of IP3 and diacyl glycerol, plays an important role in mediating αmβ3 activation. IP3-mediated increases in cytosolic-free Ca2+ appears to be required for integrin activation, but Ca2+ alone is obviously not sufficient to induce inside-out signaling. Activation of PKC by diacyl glycerol or phorbol esters leads to αmβ3 activation. It is, however, not clear which of the various PKC isoforms are involved in G protein–mediated inside-out activation and which are the relevant PKC substrates in this process. The requirement of Goq-mediated signaling for agonist-induced αqβ3 activation has also been demonstrated by the phenotype of Goq-deficient platelets, which fail to aggregate and to secrete in response to thrombin, ADP, and TxA2, because of a lack of agonist-induced PLC activation. There is, however, clear evidence for additional Gq-independent signaling processes which are involved in αmβ3 activation. This has first been suggested based on several observations made when the mechanisms of ADP-induced integrin αmβ3 activation were studied. Platelets lacking the Gq-coupled P2Y12 receptor or in which P2Y12 was blocked did not aggregate in response to ADP unless the Gi-mediated pathway was activated by P2Y12. 

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Go* show reduced aggregation in response to ADP, thrombin or adrenaline.17–20,108,109 Thus, Go, and Gq appear to synergize to induce platelet aggregation.

How Gq contributes to integrin αmβ3 activation in platelets is currently not clear. It is conceivable that the Gq-mediated decrease in cAMP levels counters the antiaggregatory effects of endothelial mediators such as PGI2, which increases platelet cAMP levels via its Go-coupled receptor. However, under in vitro conditions, a decrease in cAMP levels alone does not induce aggregation of platelets.19,100,101 Activation of Gq results in the release of reasonable amounts of G-protein βγ subunits, which, as dimers themselves, regulate various effectors and may contribute to αmβ3 activation and platelet aggregation. The 2 PI3K isoforms activated by βγ complexes, p110β (PI3Kβ) and p110γ (PI3Kγ), are both expressed in platelets.112,113 Activation of PI3K results in the formation of 3-phosphorylated phosphoinositides, which can activate a variety of effectors including various isoforms of PKC or PKB/Akt.12,14 A role of PI3K in the activation of integrin αmβ3 is also supported by observations in PI3Kγ-deficient mice, which show reduced aggregation responses to ADP,114,115 as well as by studies using specific inhibitors of PI3Kβ, which suggests that this enzyme plays an important role in sustaining platelet aggregation in response to low concentrations of platelet activators.116 Consistent with that, platelets lacking the downstream effectors of PI3K, Akt1, and/or Akt2 show reduced aggregation.118,119

Recently, the small GTPase Rap1 has attracted interest as a potential mediator of integrin αmβ3 activation by various platelet activators. Rap1, which has been implicated in the activation of inside-out signaling for a variety of integrins including αmβ3,120,121 is rapidly activated on platelet activation obviously through Gq and Gq-mediated signaling pathways.122,123 Mice lacking Rap1b show defects in the activation of integrin αmβ3 in response to various platelet stimuli and are protected from arterial thrombosis.124 Similarly, mice lacking CalDAG-GEFI, a guanine nucleotide exchange factor mediating agonist-induced Rap1 activation in platelets, show decreased platelet activation and thrombus formation and have severely prolonged bleeding times.125 These data clearly indicate that Rap1 is involved in at least 1 of several mechanisms mediating the integrin αmβ3 activation by various platelet activators.

There is also clear evidence that Gi1-mediated signaling contributes to efficient αmβ3 activation. This has first been suggested on the basis of the observation that some level of integrin αmβ3 activation and platelet aggregation can also be induced in the absence of Gi1-mediated signaling when Gq- and Gi2/Gi3-dependent signaling pathways are concomitantly activated.126,127 A role of Gi1 in platelet integrin activation could be confirmed in studies with platelets lacking Go13. In the absence of Go13, agonist-induced αmβ3 activation was reduced. In addition, Go13-deficient animals had prolonged bleeding times and were protected against arterial thrombosis.128 A role of the Gi13-mediated activation of the Rho/Rho-kinase pathway is consistent with findings that indicate a role of RhoA in platelet aggregation under high-shear conditions and in the irreversible aggregation of platelets in suspension.129

Although the precise signaling mechanisms linking GPCRs to integrin αmβ3-mediated platelet aggregation are only partially understood, it has become clear in recent years that the rapid platelet aggregation with high efficiency requires the activation of at least 3 signaling pathways mediated by the heterotrimeric G proteins Gi13, Gq, and Gq. In the absence of 1 of the 3 pathways, activation of αmβ3 still occurs, although with lower efficacy.

**Platelet Secretion and Procoagulant Activity**

Secretion from platelets is an important mechanism that amplifies platelet activation and results in the release of mediators that act on the vessel wall as well as on other blood cells. It occurs in 2 waves, the first consists of the release of dense core granules and α-granules followed by the release of lysosomes. Dense granules contain small molecules such as nucleotides (ADP, ATP) or serotonin, whereas α granules contain various proteins including growth factors, chemokines, adhesive molecules, and coagulation factors. There is good evidence that platelet secretion functions analogous to other systems of regulated secretion, and platelets have been shown to contain the necessary components of the secretory machinery.130,131 Although a detailed model of the mechanisms linking platelet surface receptors to the activation of platelet secretion is still missing, some of the upstream regulatory processes have been elucidated. It is well established that an increase in the free cytosolic [Ca2+] and an activation of PKC are required for platelet activation.76,132 Platelet secretion can be inhibited by various inhibitors of PKC isoforms.133–136 However, the identity of the PKC isoforms involved in stimulus-induced platelet secretion is not clear. PKCα has been suggested to be involved.137 Several targets of Ca2+ and PKC have been suggested including Munc-18, MARCKS proteins, or phosphatidylinositol-5-phosphate 4-kinase.138 Although elevation of cytosolic [Ca2+] and activation of PKC are able to induce some platelet degranulation, full secretory responses of platelets to various stimuli require the synergistic activation of Ca2+ and PKC-mediated processes.139–140 The central role of the Gq/PLC-β pathway in agonist-induced platelet granule secretion is supported by the finding that various platelet activators failed to induce secretion in platelets lacking Go68 and by the fact that secretion in response to TxA2 and low concentrations of thrombin are reduced in PLCβ-deficient platelets.116

Platelet-shape change does not appear to be required for platelet aggregation.141 Platelet-shape change, however, precedes platelet secretion, and contractile forces generated during platelet-shape change lead to the centralization of platelet granules. Centralization of platelet granules is believed to be required for fusion of granules with each other as well as with the open canicular system and the plasma membrane. This implies that the cytoskeletal reorganization during platelet-shape change contributes to the secretion of platelet granules. Consistent with this, signaling pathways that are involved in the induction of platelet-shape change such as the Ca2+-dependent and the RhoA/Rho-kinase–mediated stimulation of MLC phosphorylation have been shown to be involved in platelet secretion,142,143 and platelet secretion
in response to thrombin and TXA₂ is severely impaired in platelets lacking Gα₁₃.³⁰

It is well established that the activation of platelets and the activation of the coagulation cascade are complementary processes that influence each other. Platelet secretion contributes to the procoagulant activity of activated platelets by providing additional coagulation factors including factor V, factor VIII, or fibrinogen.¹⁴¹ In addition, strong platelet activation results in the exposure of phosphatidylserine at the outer surface of the plasma membrane as well as in the formation of membrane blebs and microvesicles. The exposure of phosphatidylserine supports the formation of thrombin by facilitating the assembly of the prothrombinase and tenase complexes on the surface of activated platelets.³¹,¹⁴⁵

The shedding of membrane blebs into the circulation is suggested to provide procoagulant microvesicles. There is good evidence that the procoagulant activity of platelets is induced by very strong and prolonged increases in intracellular [Ca²⁺]. The most important stimulus appears to be fibrillar collagen, which acts through primarily G protein–independent processes. The transient [Ca²⁺] increases induced via G₁₂-coupled receptors are obviously only sufficient to induce a small degree of phosphatidylserine exposure and procoagulant activity.³¹ Nevertheless, the autocrine activation of platelets by ADP and subsequent activation of P2Y₁ and P2Y₁₂ receptors contributes to the activation of platelet procoagulant activity in vitro and in vivo.²⁸,¹⁴⁶,¹⁴⁷

**Implications for Future Development of Antiplatelet Agents**

Platelet adhesion at sites of atherosclerotic plaque rupture and subsequent formation of platelet aggregates is a central mechanism underlying the formation of arterial thrombi, resulting in acute coronary syndromes, stroke, and peripheral artery disease. In addition, platelet activation appears to be already involved in earlier stages of atherosclerosis development.⁶⁰,¹⁴⁸ Platelets are therefore prime targets for therapeutic approaches to treat or prevent cardiovascular diseases.²⁸,¹⁴⁹

Although considerable progress has been made in the development of antiplatelet agents as well as in the clinical evaluation of their effects, available antiplatelet therapies are still not optimal. This topic will be covered further in a forthcoming review in this series.

The currently most widely used antiplatelet drugs, aspirin, and clopidogrel have been shown to prevent cardiovascular diseases and are relatively well tolerated by treated patients. However, despite the proven beneficial effects, both drugs can reduce the risk for serious vascular events such as myocardial infarction or stroke in high-risk patients only by approximately one-quarter.¹⁵⁰,¹⁵¹ Thus, the efficacy of aspirin and clopidogrel is limited. This is probably attributable to the fact that both drugs inhibit the action of only 1 of several positive-feedback mediators acting via GPCRs. A third class of antiplatelet drug, α₃β₁ (GPIIb/IIIa) antagonists, block the final convergence point of platelet activation. Blockers of α₂β₁ integrin have been shown in clinical trials to be very efficacious when used in patients undergoing coronary interventions. However, the increased efficacy is accompanied by a considerable reduction in safety, which restricts the use of α₃β₁ antagonists to the treatment of acute clinical conditions or to the treatment of patients undergoing coronary interventions.¹⁵²,¹⁵³ Thus, especially for the prevention of cardiovascular diseases, there is still a clinical need for antiplatelet drugs with higher antithrombotic efficacy than aspirin and clopidogrel but with safety profiles that allow for a preventive long-term administration.

Because G₉, G₁₁, and G₁₃ integrate the effects of various platelet stimuli acting through their GPCRs, interference with signaling pathways downstream of GPCRs appears to be a promising strategy to develop antiplatelet agents with higher efficacy than those that block only individual platelet stimuli or their individual receptors. Because all 3 G protein–mediated signaling pathways show some level of redundancy, inhibition of 1 of the pathways should still be safer than blocking the common end point of platelet activation. Based on the fact that platelets lack G₁₁, which can compensate G₉ deficiency in almost all tissues except platelets and the nervous system,¹⁵⁸,¹⁵⁴ G₉ has been suggested as a target for antiplatelet agents.⁶⁸ To circumvent potential side effects in the central nervous system, a G₉ inhibitor should not be able to pass the blood/brain barrier. It has, however, turned out to be extremely difficult to selectively target the function of G₉ because of its close structural similarity to Gα₁₃.¹⁵⁵ However, a G₉-specific approach would be required, because inhibition of both G₉ and G₁₁ is likely to have deleterious effects in multiple organs.¹⁵⁶–¹⁵⁸ Alternatively, signaling pathways downstream of G₉, G₁₁, or G₁₃ such as the Rho/Rho-kinase or the PI3K-mediated signaling pathways represent attractive targets for antiplatelet drugs.¹⁵⁹,¹⁶⁰ Because most components of G protein–mediated downstream signaling pathways are present in a variety of cells, potential inhibitors should act in a somewhat platelet-selective manner to reduce the risk of side effects in other cells and tissues. Depending on their pharmacokinetic and pharmacodynamic properties, drugs acting on widely expressed protein targets can have remarkable platelet selectivity, as demonstrated by the well-established antiplatelet activity of low-dose aspirin.¹⁶¹

**Conclusions**

After the initial interaction of platelets with the altered vessel wall and their adhesion, the subsequent activation and formation of a platelet thrombus require the action of various diffusible mediators that act through GPCRs. In recent years, the major GPCRs involved in platelet activation under physiological and pathological conditions have been identified, and it has become clear that platelet activation requires the parallel signaling through several heterotrimeric G proteins. Although, in the absence of G₂₅, G₂₆, or G₂₇-mediated signaling, some platelet activation can occur, efficient activation of platelets in vitro and in vivo requires all 3 G protein–mediated signaling pathways. Mouse lines lacking individual G-protein α subunits or other downstream signaling components of these pathways have provided the first models not only to evaluate the physiological role of individual signaling pathways but also to make predictions regarding their potential role as targets for new antiplatelet agents.
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References


58. Schafer A, Schulz C, Eigenthaler M, Fraccarollo D, Kobsar A, Gawaz M, Langer H, May AE. Platelets in inflammation and athero-


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