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REVIEW ARTICLE

PHYSIOLOGY OF BLOOD PLATELET ACTIVATION

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Abstract: Blood platelets interact with a variety of soluble agonists such as epinephrine and adenosine diphosphate (ADP); many insoluble cell matrix components, including collagen and laminin, and biomaterials used for construction of invasive medical devices. These interactions stimulate specific receptors and glycoprotein-rich domains (integrins and nonintegrin) on the plasma membrane and lead to the activation of intracellular effector enzymes. The majority of regulatory events appear to require free calcium. Ionized calcium is the primary bioregulator, and a variety of biochemical mechanisms modulate the level and availability of free cytosolic calcium. Major enzymes that regulate the free calcium levels via second messengers include phospholipase C, phospholipase A_2, and phospholipase D, together with adenylyl and guanylyl cyclases. Activation of phospholipase C results in the hydrolysis of phosphatidyl inositol 4, 5-bisphosphate and formation of second messengers 1,2-diacylglycerol and inositol 1,4,5-trisphosphate (IP_3). Diglyceride induces activation of protein kinase C, whereas IP_3 mobilizes calcium from internal membrance stores. Elevation of cytosolic calcium stimulates phospholipase A_2 and liberates arachidonic acid. Free arachidonic acid is transformed to a novel metabolite, thromboxane A_2, by fatty acid synthetases. Thromboxane A_2 is the major metabolite of this pathway and plays a critical role in platelet recruitment, granule mobilization and secretion. Up-regulation in signalling pathways will increase the risk for clinical complications associated with thromboembolic episodes. Down-regulation of signal transduction mechanisms may precipitate bleeding diathesis or stroke.

Key words : platelet activation signal transduction second messengers calcium arachidonic acid phosphinositides G-proteins

INTRODUCTION

Platelets play a critical role in the recognition of vascular injury, formation of effective hemostatic plugs, retraction of clots, and wound healing (1-14). However, when hyperactive, they can initiate events leading to many clinical complications associated with cerebro-cardiovascular problems. They lack deoxyribonucleic acid (DNA), and, therefore, do not synthesize major biochemical components and do not replicate (15). They come into the circulation well-packaged with the other required biochemical components and have a relatively short half life (5-6 days).

Although completely nonsticky, in the resting state they can become sticky with the slightest stimulation. The degree of activation depends on the strength of the stimulus and the information available in the interactive domains of the cell matrix components (16). The platelet is bestowed with multiple signal receiving mechanisms (17). It can revert back to the resting state if the full activation is not warranted. Four well-defined

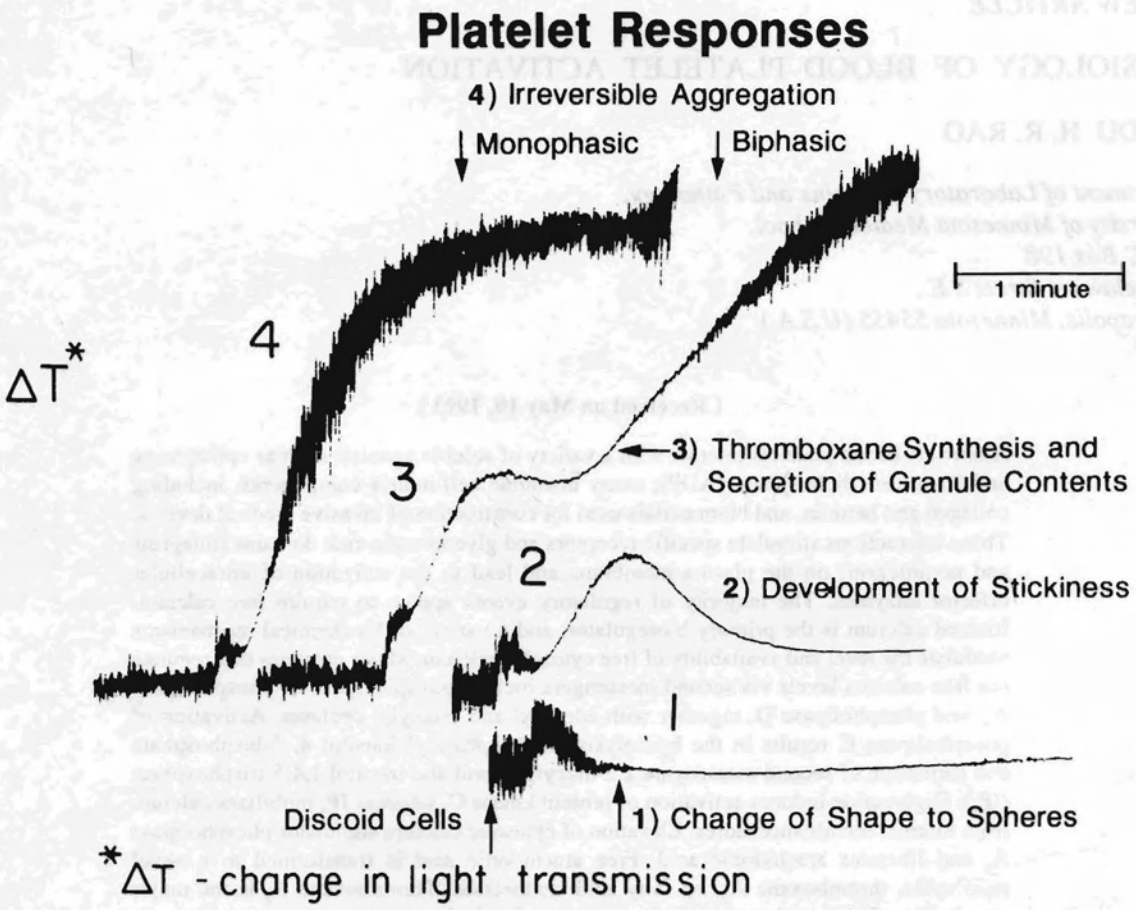

Fig. 1: Influence of various concentrations of thrombin on discrete platelet responses was followed on an aggregometer. Loss of oscillations shows change of discoid shape of platelets to spheres (1). Slight upper curve demonstrates development of stickiness and primary reversible aggregation (2). Optimum concentration of thrombin generates biphasic aggregation. Second wave of aggregation is dependent on prostaglandin endoperoxides/thromboxanes or adenosine diphosphate released from storage organelles (3). At high concentrations, thrombin causes monophasic irreversible aggregation.

stages of activation are recognized: (1) development of stickiness, (2) changes in the cell shape, (3) contraction and release of granule contents (secretion or release reaction) and (4) irreversible aggregation (18). The exact biochemical mechanisms involved in the first two phases of activation (shape change and development of stickiness) are not known. Major biochemical events associated with ligand binding, signal transduction, formation of second messengers, calcium mobilization, contraction, release of granule contents and irreversible aggregation have been described by several laboratories (19-51). In this overview some salient features of platelet morphology, biochemistry and physiology are described.

Platelet morphology and biochemistry: Human platelets have a discoid form in their resting state (52-53). In order to relate structure to function, white has divided anatomy of the platelet into distinct zones (53). The Peripheral Zone consists of membranes and closely associated structures. An exterior coat or glycocalyx is rich in glycoproteins. The middle layer of the peripheral zone is rich in phospholipids. More than 15% of the dry weight of platelets is lipid of which 80% is phospholipid (54). Major lipids include: cholesterol (30.8%), phosphatidylcholine (26.3%), phosphatidyl

ethanolamine (8.6%), sphingomyelin (11.6%), phosphatidyl serine (6.6%), and phosphatidylinositol (2.7%).

Platelet plasma membranes contain transmembrane proteins as well as glycoproteins. Glycoproteins are embedded in the lipid bilayer. They serve as the receptors for agonist/surface-mediated stimuly triggering platelet activation. Platelets contain integrin as well as non-integrin domains in their plasma membranes (55). Integrins are transmembrane glycoproteins with alpha and beta units coupled nonvovalently (GPIIb-IIIa, GPIa-IIa, GPIc-IIa). They participate in both cell-cell and cell-matrix interaction (55-58). In addition, there are nonintegrin glycoprotein domains on platelets capable of binding other proteins such as collagen and von Willebrand factor (GPIV, GPIb).

Platelets utilize energy for a variety of biochemical events associated with activation. Unlike muscle, platelets do not contain creatine phosphate or creatine phosphokinase. They contain large amounts of glycogen (30 $\mu m/10^{11}$ cells). Platelets have a prominent glycolytic cycle and relatively few mitochondria. Thus, the principal source of energy comes from the hydrolysis of ATP (59, 60).

The *sol-gel zone* is the matrix of the platelet cytoplasm. It contains fiber systems in various states of polymerization. These systems support the discoid shape in resting platelets and provide a contractile system involved in shape change, pseudopod extension, contraction and secretion. The contractile system constitutes 30% of the total platelet protein (61). A significant portion of this system is actin. Other proteins of the platelet contractile system include myosin, tropomyosin, acting binding protein, a-actinin, gelsolin, profilin, vinculin and spectrin.

The *organelle zone* consists of granules, dense bodies, peroxisomes, lysosomes, mitochondria and glycogen. This zone serves as the storage site for various enzymes, nonmetabolic adenine nucleotides, serotonin, a variety of proteins, calcium, and antioxidants such as ascorbic acid, glutathione and taurine (62, 63).

The *membrance system* plays a major role in platelet physiology. The dense tubular system (DTS) has been shown to be the site where calcium, an important bioregulator, is sequestered. The DTS is also the site where enzymes involved in prostaglandin synthesis are localized (64). The surface-connected open canalicular system (OCS) provides access to the interior for plasma borne substances and serves as a conduit for products secreted during the release reaction (65).

Platelet responses: Platelet responses can be divided into two categories - first, the reversible responses, including change of shape, development of stickiness, adhesion, and primary reversible aggregation; and second, contraction, release reaction and irreversible aggregation (Fig.1). When stimulated in suspension, platelets change shape, become sticky, aggregate if brought into contact with each other by stirring, secrete the contents of their granules-dense bodies, alpha granules and lysosomes. All these responses can be monitored on a platelet aggregometer (18).

Platelet shape change: Most stimuli cause a change in the shape of platelets. Discoidal cells, when stimulated, extend pseudopodia or filopodia from their peripheral zone, followed by change in their shape to a spiny sphere (dendritic) (24). The fine pseudopodia, formed upon stimulation, consist mainly of bundles of actin filaments complexed with profilin. In addition, tropomyosin, alpha-actin, and actin binding protein also participate in the formation of pseudopodia. In addition to soluble physiological agonists, a variety of compounds such as phorbol esters and calcium ionophores also cause aggregation (66, 67). Macromolecular components such as collagen fibrils, bacterial and tumor cells also cause platelet activation (67, 68). Change from a disc to an irregular sphere, constriction of microtubules and centralization of the granules may require phosphorylation of myosin light chain and contraction.

Platelet aggregation: Adenosine diphosphate (ADP, 3 μM), thrombin (0.2 U/ml), serotonin (5HT, 10 μM), vasopression (100 mU/ml), epinephrine (5 μM), arachidonic acid (0.4-0.6mM) and platelet activating

factor PAF, 2 μM) initiate aggregation within a few seconds. By and large, all physiological agonists induce irreversible aggregation by the mediation of newly synthesized prostaglandin endoperoxides, thromboxanes or secreted adenosine diphosphate. Two types of responses can be observed *in vitro*, a primary aggregation that is reversible and occurs without secretion and a secondary aggregation that is accompanied by the release of granule contents (62, 63). Calcium or magnesium and fibrinogen are required for agonist-mediated platelet activation leading to aggregation (71). Several studies have characterized a complex of two of the glycoproteins (GP) on the platelet surface, GPIIb and GPIIIa as the fibrinogen receptors. Specific membrane spanning receptors for agonists such as epinephrine, thrombin, and thromboxane have also been characterized on the platelet surface membrane.

Platelet adhesion: Mechanisms that underlie platelet adhesion are not clear (72). Platelet adhesion to glass and synthetic surfaces is probably mediated by fibrinogen and, therefore, depends on GPIIb-IIIa. Platelets adhere to collagen, an extracellular matrix component by different specific receptors (GPIV, GPIa-IIa) (73). There appears to be a central role for von Willebrand factor (vWF) in the adhesion of platelets to the subendothelium (74). The vWF is rich in carbohydrates and interacts with surface membrane receptor GPIb. Adhesion to natural and artificial surfaces is associated with release of granule contents and is not inhibited by the majority of anti-platelet drugs.

Platelet release reaction: The contents of three types of granules are released when platelets are activated by agonists or various natural and artificial surfaces. Weak agonists such as epinephrine, ADP and PAF depend on formation of arachidonate metabolites, prostaglandin (PG) endoperoxides (PGG_2/PGH_2) or thromboxane for causing release of granule contents. Potent agonists such as collagen, thrombin, the calcium ionophore, A23187, and the ATPase inhibitor, thapsigargin, can induce secretion of granules independent of arachidonate metabolites (75). The contens of granules are released to the surface-connected canalicular system and extruded to the outside of the cell (62, 63).

Stimulus-response-coupling: None of the physiological agonists can penetrate the plasma membrane of the platelet (18, 19). They must, therefore, react at interactive domains on the platelet surface membrane, initiate appropriate signal, convey the message across the plasma membrane to the interior and activate intracellular effector enzymes (17-22). Activation of these enzymes results in the formation of second messengers that regulate the level of available cytosolic ionized calcium (23, 28, 30-51).

Physiological agonists and components of the extracellular matrix such as collagen, fibronectin and laminin, induce platelet activation by binding at specific plasma membrane domains (receptors, integrins, nonintegrin glycoprotein-rich domains) and initiating appropriate activating signals. The exact mechanisms involved in ligand binding associated signal generation at the receptor level is not clear.

Platelet receptors: A receptor for ADP has been characterized using affinity labeled fluorosulfonylbenzyolyl adenosine (FSBA) as aggregin, a 100 Kd protein. Stimulation of this receptor induces shape change, aggregation and exposure of binding sites for fibrinogen (18, 19). Several receptors for collagen have been described. In these reports GPIa and GPIV dominate as the putative receptor sites. Thromboxane A_2 receptor has been characterized using receptor antagonists and photoaffinity labels. Thrombin receptor has been identified, although there is still considerable interest in GPV and GPIb as possible receptor sites. $Alpha_2$ adrenergic receptor has been cloned and expressed and serves as interactive domains for catecholamines (43).

Signal transduction: Receptors for epinephrine, thrombin and thromboxane are members of the G-protein receptor family. Platelets contain phosphoinositide specific phospholipase C(PLC), the activity of which is modulated by G-proteins (35, 39-51). Thrombin and thromboxane also stimulate tyrosine phosphorylation, which can also initiate tyrosine phosphorylation-dependent activation of PLC (34). Platelets contain high levels of the protein tyrosine kinase (PTK) $PP60^{C\text{-}SRC}$. Other PTKs of the SRC family, namely $PP60^{fyn}$, PP55, 58^{lyn} and $PP61^{C\text{-}YES}$ are also present in platelets. In addition to these mechanisms, platelets initiate other intracellular enzymes such as phospholipase D, phospholipase A_2 via G-protein mediation or independent of G-proteins by as yet unknown mechanisms (42).

Ionized calcium is the primary regulator of platelet activation sequences (47, 48, 67, 76-80). The level of free calcium available dictates the relative state of cell activation. In resting platelets the cytosolic-free calcium levels are maintained at appromimately 1×10^{-7} M by efficient calcium transport pumps and calcium-binding proteins (80). Physiological agonists as well as antagonists exert their effect on platelets by modulating levels of ionized calcium through a complex chain of signaling mechanisms (Fig. 2). To communicate with internal effector enzymes, agonists and antagonists act on the platelet plasma membrane at discrete sites (receptors/integrins/glycoprotein-rich domains) and generate activation and inactivation signals. These signals are then transduced across the plasma membrane to appropriate effector enzymes, such as adenylyl cyclase (AC), guanylyl cyclase (GC), phospholipase C (PLC), phospholipase A_2 (PLA_2), and phospholipase D (PLD) via GTP-binding protein (G-protein) dependent and independent mechanisms. Once the effector enzymes are activated, the appropriate second messengers are generated and these molecules further activate other enzymes or serve as substrates for the generation of additional potent bioactive metabolites.

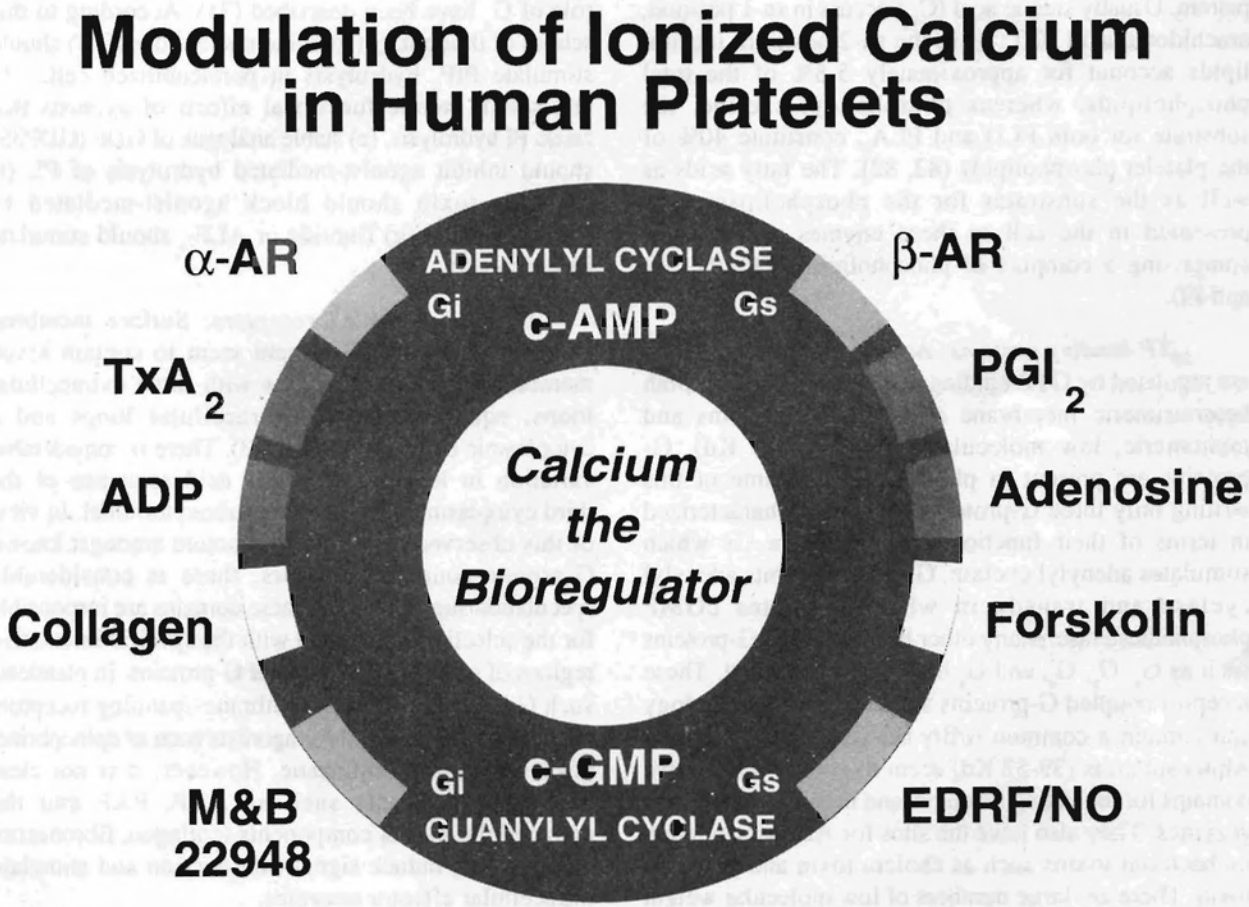

Fig. 2: Ionized calcium is the primary bioregulator. Level of cytosolic free calcium is regulated by second messengers cyclic AMP and cGMP. Agonists and antagonists act at specific plasma membrane receptors and modulate the activity of intracellular effector enzymes, adenylyl cyclase or guanylyl cyclase and regulate the level of second messengers cAMP/cGMP.

Platelet phospholipids: Major substrates for the phospholipases as well as for PI 3-kinase reside in the membranes (81-88). Usually threre is enough PLC activity to hydrolyze all the inositol phospholipids. Yet,

the enzyme is unable to get access to these substrates (PI, PIP, PIP_2). Stimulation of cell surface by agonists brings about required alterations in the membrane or the microenvironment to make these substrates available for the action of enymes. Furthermore, there seems to be some specificity as to which species of the substrates is favourably predisposed for hydrolysis. Agonist-mediated activation in platelets seems to favour the hydrolysis of PIP_2 (84-89).

Fatty acid composition of phospholipids follow a pattern. Usually stearic acid (C_{18}) occurs in sn-1 position, arachidonic acid (c20 : 4) in the sn-2 position. Inositol lipids account for approximately 5-8% of the total phospholipids, whereas phosphatidyl choline, the substrate for both PLD and PLA_2, constitute 40% of the platelet phospholipids (82, 82). The fatty acids as well as the substrates for the phospholipases are presented in the cell to these enymes as a bilayer comprising a complex of phospholipids (PC, PE, PS and PI).

GTP-binding proteins: Activity of phospholipases are regulated by GTP-binding proteins (40-43, 51). Both heterotrimeric membrane associated G-proteins and monomeric, low molecular weight (20-26 Kd), G-proteins are present in platelets. At the time of this writing only three G-proteins have been characterized in terms of their function (31). They are Gs which stimulates adenylyl cyclase, Gi which inhibits adenylyl cyclase and transducin which regulates cGMP phosphodiesterase. Many other heterotrimeric G-proteins such as G_o, G_p, G_q, and G_z have been described. These receptor coupled G-proteins share extensive homology and contain a common $\alpha/\beta/\gamma$ heterotrimeric structure. Alpha subunits (39-52 Kd) seem to contain interactive domains for regulating receptors and intracellular effector enzymes. They also have the sites for ADP ribosylation by bacterial toxins such as cholera toxin and pertussis toxin. There are large numbers of low molecular weight G-proteins in the cytosol. They are members of the ras superfamily and belong to three major groups: ras, rho, and rab.

Current concepts on the mechanism of action of heterotrimeric G-proteins envisages that occupation of the receptor by an agonist increases the rate of dissociation of GDP from the α-subunit, facilitating the binding of GTP, resulting in the dissociation of G-protein into a free α-subunit and β/γ dimer (40-43). The α-subunit with bound GTP stimulates the effector enzyme and this stimulatory activity is terminated when GTP is converted to GDP by the intrinsic GTPase activity. Alpha subunit coupled to GDP can then reassociate with the β/γ dimer.

The G-protein that initiates hydrolysis of PI by stimulating PI-specific phospholipase C is termed G_p. However, such a G-protein has not been characterized in platelets, but conditions required to demonstrate the role of G_p have been described (31). According to this school of thought, (a) guanine nucleotides (GN) should stimulate PIP_2 hydrolysis in permeabilized cells, (b) GN should mimic functional effects of agonists that cause PI hydrolysis, (e) stable analogue of GDP (GDPβS) should inhibit agonist-mediated hydrolysis of PI, (f) pertussis toxin should block agonist-mediated PI hydrolysis, and (g) fluoride or $ALF-_4$ should stimulate PI hydrolysis.

G-protein-coupled receptors: Surface membrne receptor coupled to G-protein seem to contain seven membrane spanning domains with three extracellular loops, equal number of intracellular loops and a cytoplasmic carboxyterminal (43). There is considerable variation in length and amino acid sequence of the third cytoplasmic loop and the carboxyterminal. In view of this observed variation in structure amongst known G-protein-coupled receptors, there is considerable speculation suggesting that these domains are responsible for the selective interaction with the specific interactive regions of regulatory subunits of G-proteins. In plateletes such G-protein-coupled, membrane-spanning receptors have been characterized for agonists such as epinephrine, thrombin, and thromboxane. However, it is not clear how other agonists such as ADP, PAF and the extracellular matrix components (collagen, fibronectin, and laminin) induce signal transduction and stimulate intracellular effector enzymes.

Phospholipid metabolism: There are four major phospholipid species associated with platelet membranes (54). They are phosphatidyl inositol, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine. Several types of phospholipases can hydrolyze phospholipids from cell membranes. Phospholipase A_1 specifically removes the fatty acids from the carbon 1 position, whereas phospholipase A_2 exerts its effect on

fatty acids attached to the second carbon of the phospholipids. Phospholipase C liberates phosphorylated bases from the phospholipids, whereas phospholipase D releases the bases such as inositol, choline, ethanolamine, and serine from the phospholipids.

In human platelets various stimuli that induce activation stimulate phospholipase C, which is closely coupled to specific receptors or sites of action on the plasma membrane. Platelet membranes contain large amounts of phosphatidyl inositol (PI) and relatively smaller amounts of phosphatidyl inositol-4 phosphate (PIP) and phosphatidyl inositol 4, 5-bisphosphate (PIP_2) (82, 83). All three species can serve as substrates for the action of phospholipase C. However, physiological agonists seem to cause hydrolysis of PIP_2 which liberates two putative messengers, 1, 2-diacyl glycerol and inositol 1, 4, 5-trisphosphate (Fig. 3). If all three species are hydrolyzed, then three molecules of diglyceride are formed for every one molecule of inositol 1, 4, 5-trisphosphate (IP_3) generated. Diglyceride produced could be further metabolized to form phosphatidic acid via the action of specific kinases or acted on by a diglyceride lipase and a monoglyceride lipase release free fatty acids (82-89). Diglyceride may also play a critical role in activation of the multifunctional enzyme protein kinase C, whereas IP_3, which is hydrophilic, mobilizes ionized calcium from internal membrane storage sites (17).

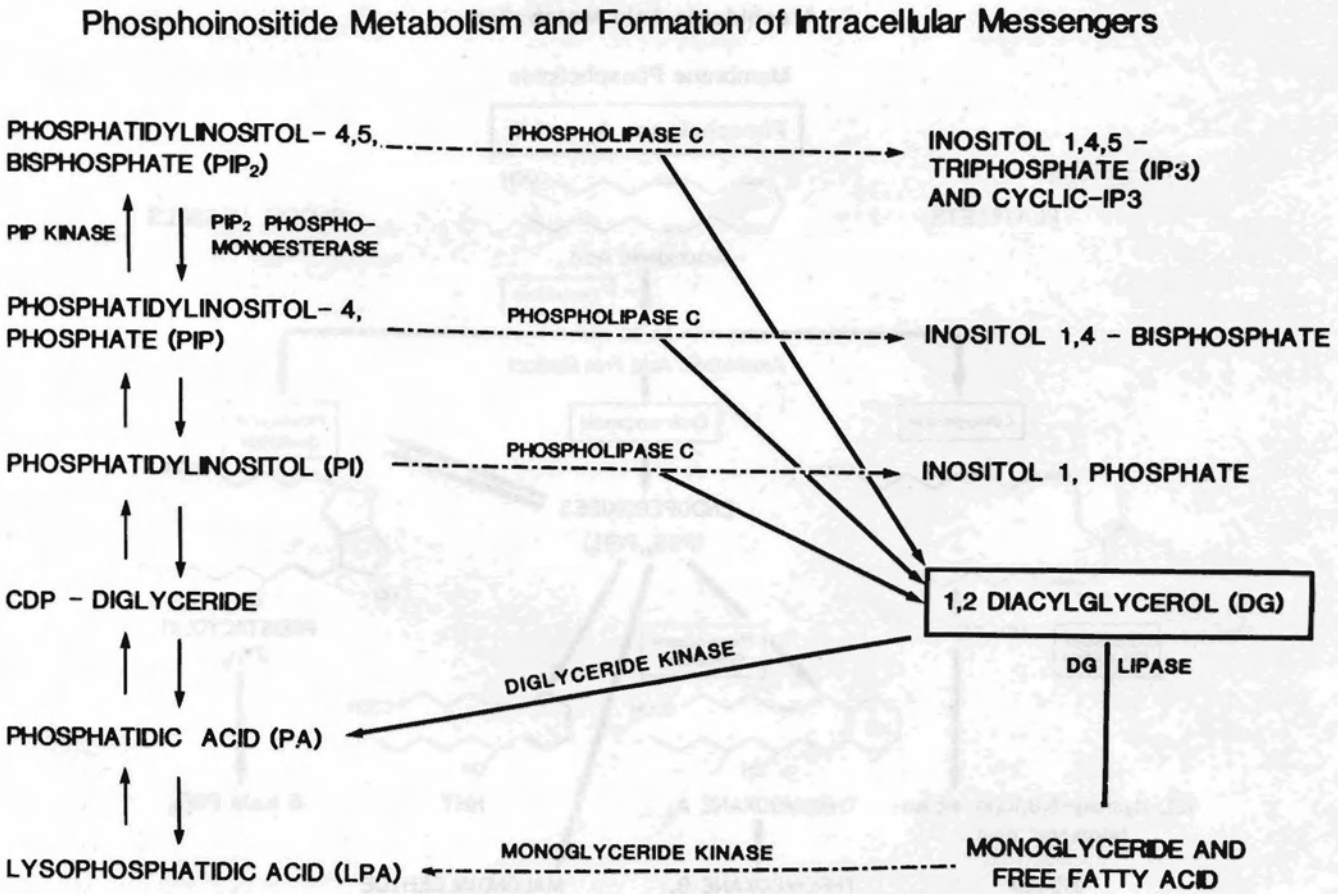

Fig. 3: Agonist-mediated activation via GTP-binding proteins results in the hydrolysis of phosphatidyl inositol 4,5-bisphosphate and formation of second messengers, 1,2-diacyl glycerol (DG) and inositol 1,4,5-trisphosphate (IP_3). Diglyceride induces translocation and activation of protein kinase C, whereas IP_3 mobilizes ionized calcium from internal membrane storage sites.

Calcium metabolism: Ionized calcium is the primary bioregulator (77-80). Resting platelets have less than 100 nM cytosolic free calcium (80). Based on the observation made with the use of the calcium ionophore, A23187, researchers have suggested a critical role for calcium in platelet contraction, secretion and irreversible aggregation (67, 78). Recent studies have provided further support for these earlier observations (17, 30, 33, 37). With the availability of calcium-specific fluorophores, intracellular calcium transients can be monitored. Using such probes, several studies have demonstrated agonist-mediated elevation in cytosolic calcium (28, 33). Furthermore studies with agonist-mediated PI hydrolysis suggest that signal transduction from receptor to the intracellular effector enzymes is coupled to the formation of second messengers such as DG and IP_3 (37-51). Elevation of cytosolic calcium seems to promote a variety of Ca^{2+} -dependent events including the activation of phospholipiase A_2.

Arachidonic acid metabolism: In addition to phosphatidyl inositols, phosphatidyl ethanolamine, and phosphatidyl choline may serve as a source for arachidonic acid (88). Phospholipase A_2 is a membrane associated enzyme responsible for the liberation of arachidonic acid from these phospholipids (84, 86). Although it is capable of releasing several fatty acids from the second carbon of the phospholipids, by and

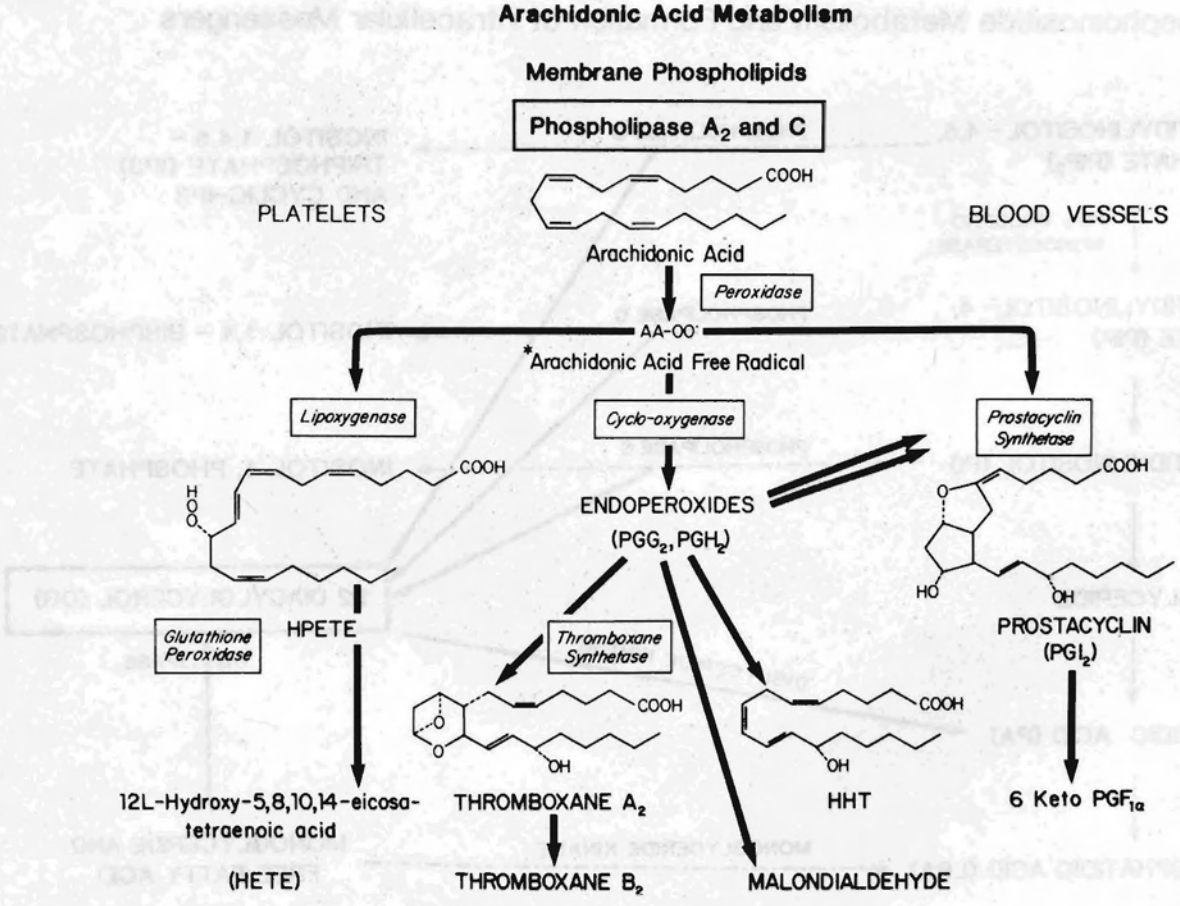

Fig. 4: Elevation of cytosolic free calcium activates phospholipase A_2. Activation of this enzyme results in the liberation of arachidonic acid (AA) from membrane phospholipids. Free AA is converted by cyclooxygenases to cyclic endoperoxides, PGG_2 and PGH_2. These transient metabolites are further transformed to thromboxane by thromboxane synthetase. In addition to this pathway, AA is converted to 12-hydroperoxyeicosatetraenioc acid (12- HPETE) and 12-hydroxyeicosatetraenoic acid (12-HETE) by lipoxygenase.

large agonist-induced stimulation of platelets seems to preferentially yield arachidonic acid.The mechanisms by which phospholipase C and phospholipase A_2 exert their specific effect on various phospholipids associated with the platelet membranes is not clear (85). Free arachidonic acid liberated during cell activation is the major substrate for the synthesis of eicosanoids (88).

Arachidonic acid released during agonist-induced activation is rapidly converted to cyclic prostaglandin endoperoxides (PGG_2/PGH_2) by cyclooxygenases and to 12-hydroperoxy acid (HPETE) and 12-hydroxy acid 12-HETE by lipoxygenase (Fig. 4). Acetylsalicylic acid (aspirin) irreversibly inhibits cyclooxygenase. Endoperoxides which are transient intermediates are as potent as thromboxane in causing platelet activation. However, these metabolites are further transformed to thromboxane A_2 by thromboxane synthetase. Thromboxane is the major metabolite of this synthetic pathway (88). In the vascular tissues prostaglandin endoperoxides (PGG_2/PGH_2) are converted to prostacyclin (PGI_2) by prostacyclin synthetase. Thromboxane is a potent vasoconstrictor and platelet agonist, whereas prostacyclin is a powerful vasodilator and platelet antagonist. These two vasoactive metabolites oppose each other's pharmacological actions by modulating the activity of adenylyl cyclase and the intracellular level of the second messenger, cAMP. This second messenger, in turn, modulates the available levels of the primary regulator, ionized calcium (17, 18, 20, 21).

Circulating adhesive proteins such as fibrinogen, components of the basement membrane, bacterial membrane proteins, certain tumor cells, and natural and artificial surfaces also interact with the platelet plasma membrane at discrete domains (integrin, nonintegrin). This results in activation and stimulation of various effector enzymes. Formation of second messengers, DG and IP_3, and various arachidonic acid metabolites (PGG_2, PGH_2, TXA_2), plays a critical role in platelet activation (development of stickiness, change of shape, adhesion, formation of binding sites for adhesive proteins, irreversible aggregation, and secretion of granule contents). Specific mechanisms involved in the process of centralization of granules and release of their contents are poorly understood.

Altered stimulus-response coupling: Several investigators have attempted to correlate the *in vitro* functional response of platelets to clinical manifestation of thrombotic episodes or bleeding diathesis (7, 8, 11, 12). Yet, it is not clear what functional responses of platelets are critical for normal hemostasis. The availability of functional glycoprotein Ib and IIb-IIIa receptors, ability to change shape, spread and become sticky, irreversibly aggregate, or release granule contents, are considered essential for effective hemostatic function. It has been shown that drug-induced impairment of signal transduction leading to altered cytosolic calcium mobilization prevents agonist-mediated secretion of granule contents and clot retraction (33). However, unavailability or lack of significant elevation of cytosolic calcium does not seem to prevent platelet shape change, development of stickiness, fibrinogen binding, adhesion or interaction with the vascular subendothelium.

Action of weak agonists for mediating secretion of granule contents depends upon the availability of thromoboxane A_2. However, bovine and equine platelets, which do not aggregate in response to the action of the arachidonate metabolite, thromboxane, support normal *in vivo* hemostasis in these animals. Similarly, the majority of dogs have platelets that do not respond to arachidonate stimulation. On the other hand, epinephrine exposure restores the sensitivity of these refractory platelets so they aggregate in response to the action of arachidonate. These observations led to the development of a concept explaining the role of catecholamines in membrane and platelet function (89).

This concept was further supported by the finding that platelets devoid of detectable cyclooxygenase activity supported normal hemostasis in adult individuals. Investigations from several laboratories have demonstrated the potentiating role of adrenalin and noradrenalin. Some reports suggest that adrenalin stimulation of alpha afrenoreceptors may amplify or restore signal transduction mechanisms.

Studies by Scrutton et al and by Storomorken and his colleagues have shown a compromised response of platelets to adrenalin in apparently normal individuals (90, 91). Weiss et al have described secretion defects in platelets from patients with bleeding tendency (92).

White and associates have followed compromised agonist response of platelets of patients with diabetes, as well as those with Hermansky-Pudlak syndrome, whose platelets lack calcium-rich dense bodies (93). Studies from our laboratory and those of others have demonstrated hypersensitivity of platelet of patients with diabetes to the action of agonists. In addition, in streptozotocin-induced diabetic rats altered vascular production of prostacyclin and platelet synthesis of thromboxane was demonstrated (94). However, the changes observed in platelet and vascular metabolism of arachidonic acid metabolism was normalized by islet tissue transplantation, suggesting a disease-specific effect (94). Contrary to the hyperactive state of platelet response reported for diabetic patients, platelets of patients with HPS exhibit compromised response to the action of agonists. This observed diminished response to agonists is attributed to the absence of dense bodies, that contribute serotonin, ADP, and calcium to the release reaction. In addition, when stimulated with weak agonists such as epinephrine, HPS platelets release significantly less arachidonic acid. Studies by Hardisty et al and Ware and associates have demonstrated altered signal transduction mechanisms and suggested impaired agonist-mediated calcium mobilization as the chief causes of platelet dysfunction (95, 96).

A brief review of the literature related to platelet disorders demonstrates a lack of detailed information in the areas of signal transduction, second messengers and calcium metabolism. Investigations on both human and animal models show that platelets can support *in vivo* hemostasis effectively in spite of a lack of some of the essential components of signalling pathways observed in human cells. Therefore, it is reasonable to conclude that platelets have multiple signalling pathways to regulate their discrete physiological responses.

However, loss or compromise of any signal mechanism may diminish their overall response to a given stimulus. For instance, it has been shown in a recent study that membrane phospholipid alterations may inactivate alpha$_1$ adrenoreceptor function (97). Modulation of this receptor plays a role in phosphatidyl inositol (PI) hydrolysis. Therefore, compromised function of this receptor will impair PI metabolism. Similarly, studies in our laboratory have shown that exogenously added phospholipase A_2 may adversely affect the platelet response to epinephrine (98). Alpha$_2$ adrenoreceptor function mediates catecholamine responses. Alteration in this receptor function may compromise the mechanism of membrane modulation.

Recent studies have shown that in many malignant and inflammatory disorders plasma phospholipase A_2 activity is elevated (99). Therefore, it is possible that various disease processes, oxidative stress and the normal aging process may contribute to the alterations in cell membrane phospholipids as well as signal transduction pathways. Further detailed studies on all aspects of signal transduction mechanisms, second messenger systems and calcium procurement pathways in both human disease models and novel animal models should be explored to elucidate specific roles of these important biochemical events in facilitating agonist-mediated discrete functional responses in platelets (100).

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