A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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ACTIVATION OF BLOOD PLATELETS AND PROSTAGLANDIN BIOSYNTHESIS

This article summarizes recent findings concerning the activation of mammalian platelets (adhesion, aggregation and secretion). Special attention is given to the metabolism of platelet arachidonic acid and production of prostaglandin and thromboxane A_2. Recent views on the role of arachidonic acid metabolites in the mechanisms of platelet aggregation, especially platelet-vessel wall interactions, are presented.

Introduction

Mammalian blood platelets are the fragments bone marrow cells called megacaryocytes. They are nonnucleated smallest blood cells, approximately 2-3 μ in diameter and about 7 μ^3 in volume.

Blood platelet participate in haemostatis, arterial thrombosis, activation of plasma coagulation, maintenance of vascular integrity and may also contribute to atherogenesis and inflammatory process. Their physiological and pathological functions are related to their ability tc adhere, aggregate and release their granule contents.

The blood platelet normally circulates as a disc and contains a number of different granules, mainly α-granules and very dense bodies. When stimulated, it changes the shape, aggregates and releases the granule contents. This action is initiated by a variety of different agents including: adenosine diphosphate, thrombin, collagen, serotonin, adrenalin and thromboxane A_2.

Activation of blood platelets by different stimuli consists of morphological and functional changes which follow a membrane signal due to the interaction of surface receptors with specific substances what leads to a sequential display of certain measurable responses of these cells. The primary stimulus for platelet aggregation appears to involve membrane glycoprotein receptors and the first response is a change in cell shape, from a discoidal to a spherical form. This is followed by the second response - aggregation, in which individual platelets form aggregates. This phase can be reversible.

A number of experiments demonstrated that fibrinogen binding to platelets is important for platelet aggregation (B e n n e t t, V i l a i r e [3]; G r a b e r, H a v i g e r [13]; M a r q u e r i, P l o w [23]; M a r q u e r i, P l o w, E d i n g t o n [22]). Fibrinogen receptor exposure on the platelet membrane and fibrinogen receptor interaction are the most critical events in platelet aggregation induced by many different stimuli such as ADP, thrombin, adrenalin, collagen, arachidonic acid and prostaglandin endoperoxide analogues. The in vitro aggregation of platelets is presumably a reflection of their major functions, the formation of the primary haemostatic plug. The photometric measurement of platelet aggregation (B o r n [4]) is by far most widely used parameter of in vitro platelet function.

Some stimuli induce a slight aggregation, called primary aggregation, followed in 1-2 minutes by a second wave of aggregation called secondary aggregation. Parallely with the second wave of platelet aggregation the platelet secretion, i.e. platelet release reaction occurs. The next responses comprising cellular synthesis of prostaglandins and secretion of substances stored in the dense bodies and α-granules are not clearly understood yet.

These events represent an ordered sequence of all responces which are due to a common intracellular messenger. The induced membrane alterations lead to the same sequential biochemical reaction and result in a rise of free Ca^{2+} ions in the cytoplasm (D e t w i l e r et al. [9]). The Ca^{2+} plays a central role in platelet activation and may be a second messenger involved in the transmission of the signal from the plasma membrane to the

platelet. Ca^{2+} mobilized from intracellular stores, most probably from the dense tubular system and membrane-bound sites (B r a s s, S h e t t i l [5]). The increase of the free Ca^{2+} ion concentration gives the typical morphological changes associated with the activation of contractile system: Ca^{2+} the common messenger for the platelet response. The inhibitory effect of cyclic AMP on platelet aggregation can be explained partly by a decrease of Ca-concentrations in the platelet cytoplasm (G o r m a n et al. [12]). On the other hand, cyclic AMP prevents exposure of the fibrinogen receptors on platelet membrane (G r a b e r, H a w i g e r [13]).

Arachidonic acid metabolism

Activation of blood platelets induced by several physical and chemical stimuli is accompanied by the synthesis of prostaglandins and prostaglandin-like compounds which play an important role in platelet function (A l l y, H o r r o b i n [1]; G e r m a n [11]; H a r l a n, H a r k e r [18]; M a l m s t e n [19]; M a r c u s [20]; M a r c u s [21]; M o n c a d a, A m e z o u a [25]; P i k e et al. [29]).

Prostaglandins are not stored but rapidly synthetized and released from cells following appropiate stimulation. Twenty carbon polyunsaturated fatty acids esterified to membrane phospholipids are their precursors. These fatty acids are either obtained directly from the diet or from elongation of the essential fatty acid, linoleic acid (G a l l i et al. [10]). In platelets and endothelial cell membranes the twenty carbon C : 20 : : 4 fatty acid with four double bonds, eicosotetraenoic acid (arachidonic acid) is the precursor of the prostaglandins containing two double bonds.

The developments in the prostaglandin field in the last few years have substantially enlarged our knowledge of the platelet - vessel wall interactions, haemostasis and thrombosis. However, the expansion of the variety of products derived from the enzymatic oxygenation of arachidonic acid has become exceedingly complex.

There are two main oxygenation pathways of arachidonic acid in platelet - the first one, based on the cyclooxygenase, which leads via the cyclic endoperoxides PGG_2 and PGH_2 to the prostaglandins and thromboxane A_2 and - the second one the lipooxygenase pathway which leads via the first formed hydroperoxy fatty acid such as 12-L-hydroperoxy, 5,8,10,14-eicosatetraenoic acid (HPETE), to a number of futher transformation products including hydroxy fatty acid 12-L-hydroxy, 5,8,10,14-eicosatetraenoic acid (HETE) and to recently discovered series of compounds - the leukotriens (N u g t e r e n [28]). If the cyclooxygenase pathway is blocked, for instance by drugs (aspirin), platelet arachidonic acid may be metabolized in the lipooxygenase pathway and produce large quantities of HETE.

Blood platelets contain very little free arachidonic acid and thus regulation of prostaglandin synthesis in platelets must occur at the level of arachidonic acid supply. When platelets are stimulated by aggregating agents, arachidonic acid is liberated. Once liberated it is rapidly metabolized via one of two pathways. Two phospholipases liberating arachidonic acid from membrane phospholipids have been identified in platelet. Their activities are stimulated by most aggregating agents caueing the increase of Ca^{2+} ion concentration. Phospholipase A_2 is a membrane-bound lipase which cleaves arachidonic acid from membrane phosphatidylcholine and phosphatidylethanolamine (V e r s t r a e t e [34]), B e l l et al. [2] provided evidence that the mechanism for arachidonic acid release from stimulated platelets involves not only phospholipase A_2 but a phosphatidylinositol specific phospholipase C liberating a digliceride from which in turn arachidonic acid is released by a membrane-bound digliceride-lipase.

In the cyclooxygenase pathway arachidonic acid is oxygenated by cyclooxygenase to labile cyclic prostaglandin endoperoxide PGG_2 with its subsequent reduction to PGH_2. The cyclic endoperoxides are intermediates with a half-life of about 5 minutes (R a z et al. [30] which may be spontaneously converted to a 17-carbon compound 12-L-hydroxy, 5,8,10-heptadecatrienoic acid (HHT) with the release of malonyldialdehyde (P o r t e r [31]). The majority of platelet endoperoxides is converted by the enzyme thromboxane synthetase to the thromboxane A_2, a highly unstable compound (H a m m a r s t r o m, F a l a r d e a u [16];

H a m b e r g et al. [15]. It is rapidly converted to biologically inactive end product, thromboxane B_2, formed nonenzymatically by incorporation of a molecule of water into TxA_2. Thromboxane A_2 has a short half-life, approximately 30 seconds in aqueous solution and about 5 minutes in plasma (N e e d l e m a n et al. [27]).

The cyclic endoperoxides may also undergo nonenzymatic conversion to small amounts of the stable prostaglandins, PGE_2, PGF_2 but primarily PGD_2 (H a m b e r g et al. [15]; M a l m s t e n [19]. Prostaglandin endoperoxides and thromboxane A_2 are extremely potent platelet aggregating agents. The mechanism by which prostaglandin endoperoxides cause platelet aggregation is not yet well understood. These compounds being unstable it is difficult to evaluate their mechanism of action. It was found that PGH_2 could directly cause aggregation of platelets and expose fibrinogen receptors on gelfiltrated platelets; the occupation of prostaglandin endoperoxide receptors on platelet surface is required for the interaction of fibrinogen and platelets (M o r i n e l l i et al. [26]). Although the mechanism of TxA_2 action in platelet aggregation has not been full understood, it appears to be involved in the regulation of intraplatelet cyclic AMP level. TxA_2 does inhibit cyclic AMP accumulation (G o r m a n et al [12]).

Contrary to platelet, in vessel wall free arachidonic acid is rapidly metabolized to cyclic prostaglandin endoperoxides and futher to prostacyclin which is the major metabolite of arachidonic acid in the vessel wall cells such as endothelial cells (G r y g l e w s k i [14]). Some amounts of PGH_2 are converted to the stable protaglandins, mainly PGE_2.

Prostacyclin (PGI_2) is a labile compound. It has a half life of about 3 minutes in aqueous solution and slightly longer in plasma. It is hydrolysed to the stable but inactive breakdown product 6-keto PGF_1 in vitro. In vivo significant amounts of other metabolites are also produced including dinor 4-keto-7,9,13-trihydroxy-prosta-11,12, enoic acid and dinor 4,13,diketo-7,9--dihydroxyprostan-1,18-dioic acid (P i k e et al. [29]).

Prostacyclin stimulates adenylate cyclase to platelets and is therefore a potent antiaggregatory agent (G o r m a n [11]; G r y g l e w s k i [14]; M o n c a d a, V a n e [24]; H a-

w i g e r et al. [17]). PGI_2 is also an inhibitor of fibrinogen binding by platelets (H e w i g e r et al. [17]). This inhibitory effect seems to be due to enhanced CAMP level which prevents exposure of fibrinogen receptor. The vessels produce much more PGI_2 than other prostoglandis. Recently the production of thromboxane A_2 by vessel wall was also observed (A l l y, H o r o b i n [1]). The small amounts of TxA_2 produced by vessel wall may be important for function regulation and play a critical role in vessel physiology and pathology.

The role of arachidonate metabolites in platelet-vessel wall interaction

Prostacyclin produced by vessel wall is a vasodilator. It not only prevents platelets from aggregation in platelet rich plasma but also dissipates preformed platelet clots and circulating platelet aggregates in vivo. Due to its properties PGI_2 was used in clinical trials as an antithrombotic agent (G r y g l e w s k i [14]).

Arachidonate metabolites, especially the unstable metabolites TxA_2 and PGI_2 modulate many of the complex platelet-vessel wal reactions (G o r m a n [11], H a r l a m, H a r k e r [18]; M o n c a d a, V a n e [24]; M o n c a d a, A m e z o u e [25]). Platelets are normally non-reactive to intact vascular endothelium. At a site of vessel injury platelets adhere to subendothelium, aggregate and develop a procoagulant activity which catalyses the intrinsic blood coagulation pathway. Platelets are thought to accelerate coagulation by thrombin generation. Vessel injury initiates platelet adherence with plasma cofactor (von Willebrands factor). Activated platelets release the active substances including ADP, which together with produced TxA_2 and thrombin cause futher platelet aggregation and release of granule contents. Upon stimulation blood platelets not only release the vasoconstractile compounds - serotonin but also synthetize the vasoconstractile PGG_2, PGH_2, TxA_2 and PGF_2 through the arachidonic acid cascade. The relaxing prostaglandins such as PGE_2 and PGD_2 are also produced (Fig. 1).

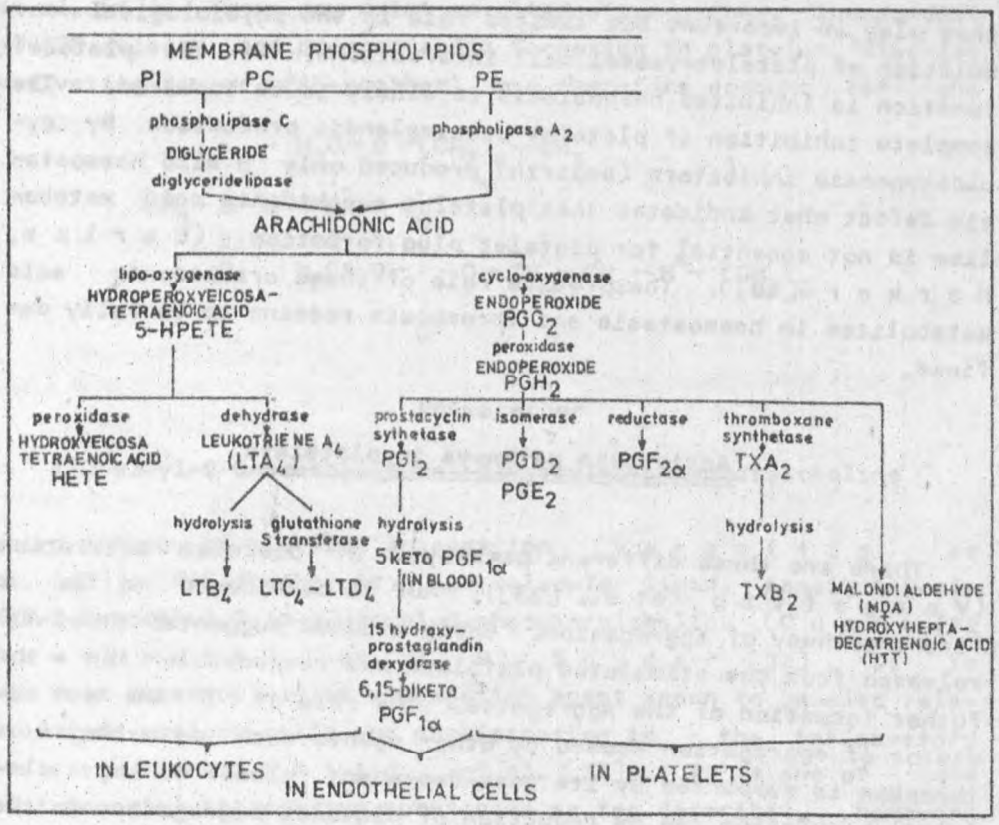


Fig. 1. Archidonic acid metabolism
Metabolizm kwasu archidonowego

Thrombin converts fibrinogen to fibrin to stabilize the platelet mass. Prostacyclin synthetized by the vessel wall in response to thrombin limits thrombus formation by inhibiting futher platelet aggregation.

It has been postulated that normal haemostasis represents a balance between platelet TxA_2 formation and vessel wall PGI_2 production. The unbalance between the production of both the compounds could lead to thrombosis or bleeding (P i k e et al. [29]).

PGI_2 and TxA_2 were at first enthusiastically introduced in many aspects of haemostasis and thrombosis (M a r c u s [20, 21]; M o n c a d a, V a n e [24]). It is apparent now that

they play an important but limited role in the physiological modulation of platelet-vessel wall interactions. If the platelet function is inhibited haemostasis is likely to be impaired. The complete inhibition of platelet prostaglandin production by cyclooxygenase inhibitors (aspirin) produced only a mild haemostatic defect what indicates that platelet arachidonic acid metabolism is not essential for platelet plug formation (H a r l a m, H a r k e r [18]). The precise role of these arachidonic acid metabolites in haemostasis and thrombosis remains to be fully defined.

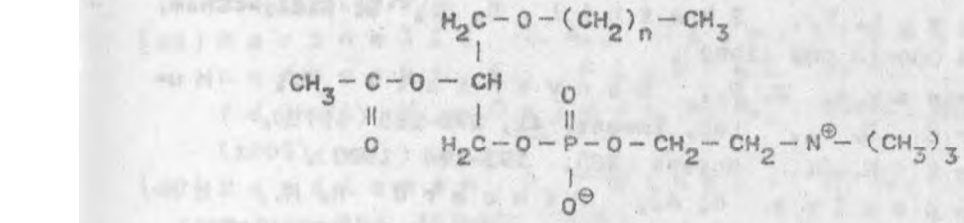
Activation pathways in platelets

There are three different pathways of platelet activation (V a r g a f t i g et al. [33]). ADP is considered to be a firs t-pathway of aggregation. Early studies suggested that ADP released from the stimulated platelets was responsible for the futher formation of the aggregates. The role of ADP as a mediator of aggregation caused by other agents such as collagen or thrombin is supported by its dose-dependent release during platelet stimulation and by reduction of platelet aggregation in the presence of ADP scavenger. The presence of ADP is required for the exposure of platelet surface fibrinogen receptors (B e n n e t, V i l a i r e [3]). The stimulated platelets release ADP and fibrinogen. Subsequently ADP acts on platelet membrane to expose fibrinogen receptors. Fibrinogen then binds to the exposed receptors what in turn results in platelet aggregation and futher activation.

The arachidonic acid cascade in presently regarded as a second pathway for aggregation mediated by produced in platelet TxA_2 (V a r g a f t i g et al. [33]; V e r s t r a e t e [34]).

Since platelet activation by thrombin or collagen is neither suppressed by the exhaustion of ADP from the granules nor inhibited by aspirin and other inhibitors of platelet prostaglandin biosynthesis, the theory was put forward that another mediator of platelet activation is formed in these cells (C a z e n a v e

et al. [6]). There may exist an alternative pathway independent of ADP release and prostaglandin formation in platelet. Platelet activating factor (PAF-acether) can therefore account for the



PAF-acether

1-O-alkyl-2-O-acethyl-2 sn -glyceryl-3-phosphorylcholine

third pathway of platelet aggregation (V a r g a f t i g et al. [33]). PAF-acether is a low molecular lipid, namely 1-O-alkyl-2-O-acethyl,2 sn-glyceryl-3-phosphorylcholine (C u s a k [7]; D e m o p o u l o s et al. [8]; S n y d e r [32]. It is the most potent platelet aggregating agent known to be also released from various cells and participating in the inflammatory process (V a r g a f t i g et al. [33]). It is one of the most powerful aggregating substances so far described, however, the mode of release of PAF-acether and the mechanism of its action is still unknown.

In activation of platelets, synergism between the action of different agents should also be taken into account.

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AKTYWACJA KRWINEK PŁYTKOWYCH I SYNTEZA PROSTAGLANDYN

Przedstawiono aktualne poglądy dotyczące aktywacji krwinek płytkowych ssaków (adhezja, agregacja, sekrecja). Zwrócono uwagę na metabolizm kwasu arachidonowego w płytce i wytwarzanie tromboksanu A_2. Przedstawiono rolę tromboksanu A_2 i prostacykliny w mechanizmach procesu agregacji, a przede wszystkim w interakcji płytki i ściany naczynia krwionośnego.