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REVIEW

Lipid Second Messengers and Cell Signaling in Vascular Wall

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Abstract—Agonists of cellular receptors, such as receptor tyrosine kinases, G protein-coupled receptors, cytokine receptors, etc., activate phospholipases (C_γ, C_β, A_2, D), sphingomyelinase, and phosphatidylinositol-3-kinase. This produces active lipid metabolites, some of which are second messengers: inositol trisphosphate, diacylglycerides, ceramide, and phosphatidylinositol 3,4,5-trisphosphate. These universal mechanisms are involved in signal transduction to maintain blood vessel functions: regulation of vasodilation and vasoconstriction, mechanical stress resistance, and anticoagulant properties of the vessel lumen surface. Different signaling pathways realized through lipid second messengers interact to one another and modulate intracellular events. In early stages of atherogenesis, namely, accumulation of low density lipoproteins in the vascular wall, cascades of pro-atherogenic signal transduction are triggered through lipid second messengers. This leads to atherosclerosis, the general immuno-inflammatory disease of the vascular system.

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In normal vessel every aspect of cell function including vascular tone regulation, metabolism and proliferative status, cytoskeleton organization, gene expression, and after all cell survival depends on external signal molecules which are either soluble hormones or proteins of extracellular matrix. These molecules act via binding with receptors on the cell surface. Upon aggregation and autophosphorylation, the receptors interact with intracellular targets via physical protein–protein interaction, namely, the attachment of SH domains, including phospholipases, to phosphorylated tyrosine, threonine, and other amino acid residues of the activated receptors. This activates kinases and phospholipases, which phosphorylate and cleave membrane phospholipids with production of lipid second messengers [1-11] (Table 1 and Fig. 1). The lipid cleavage also results in generation of active ligands that act through their own receptors. These ligands include lysophosphatidylcholine, lysophosphatidic acid, eicosanoids, and sphingosine-1-phosphate (S1P).

Further, we shall consider the generation and signal transduction mechanisms of lipid second messengers involved in signal transduction from the receptor to the transcription apparatus in the nucleus, as well as their involvement in the regulation of blood vessel functioning.

Vascular Tone

Intimal endothelium and medial smooth muscle act as contiguous tissues with tight spatial and functional

Abbreviations: AT_1) angiotensin II receptor; $cPLA_2$) cytosolic phospholipase A_2; DAG) diacylglycerides; eNOS) endothelial NO synthase; ERK) extracellular signal-regulated kinases; ET-1) endothelin-1; FAK) focal adhesion kinase; HDL) high density lipoproteins; IP_3) inositol trisphosphate; LDL) low density lipoproteins; MAPK) mitogen-activated protein kinases; MLC) myosin light chains; oxLDL) oxidized LDL; PDGF) platelet-derived growth factor; PI3K) phosphatidylinositol 3-kinase; PKB) protein kinase B/Akt; PKC) protein kinase C; PLA_2) phospholipase A_2; PLD) phospholipase D; PtdIns(3)P) phosphatidylinositol 3-monophosphate; PtdIns(3,4)P_2) phosphatidylinositol 3,4-bisphosphate; PtdIns(3,5)P_2) phosphatidylinositol 3,5-bisphosphate; PtdIns(4,5)P_2) phosphatidylinositol 4,5-bisphosphate; PtdIns(3,4,5)P_3) phosphatidylinositol 3,4,5-trisphosphate; ROS) reactive oxygen species; SAPK) stress-activated protein kinase; SMC) smooth muscle cells; S1P) sphingosine-1-phosphate; TGF-β) transforming growth factor-β; TNF-α) tumor necrosis factor-α.

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Table 1. Lipid second messengers

Second messenger	Generation pathway	Specific signal	Physiological response	References
IP_3, DAG	Hydrolysis of PtdIns(4,5)P_2 by phospholipases C_β and C_γ	Activation of G protein-coupled receptors by hormones, activation of receptor tyrosine kinases by growth factors	Vasoconstriction, vasorelaxation, proliferation, migration	[1-3]
PtdIns(3,4,5)	Phosphorylation of PtdIns(4,5)P_2 by PI3K	Cytokines, ligands of surface antigens	Cell survival	[4, 5]
Ceramide	Hydrolysis of sphingomyelin by neutral, Mg^{2+}-dependent, and acidic sphingomyelinase	Cytokines, growth factors, antibodies to CD molecules, oxLDL, thermal shock, hypoxia/reperfusion, UV radiation, drugs, lipopolysaccharides	Apoptosis, endothelial dysfunction	[6]
Sphingosine	Hydrolysis of ceramide by neutral ceramidase	Second messenger function is not established unambiguously	Arrest of cell division, apoptosis	[7, 8]
S1P	Phosphorylation of sphingosine by sphingosine kinase	Second messenger function is not established unambiguously	Cell growth, cell survival	[6, 9-11]

coordination in response to tonus and input from the bloodstream [12-14]. Vascular endothelium is the major determinant for the maintenance of vascular tone and other functions. Normal endothelium constantly releases vasoconstrictive and vasodilating stimuli in response to various influences, which regulate the vascular tone. Endothelium also regulates platelet aggregation, thrombogenesis, and proliferation/remodeling of blood vessels [15].

Vasodilation. Various receptor-dependent agonists such as acetylcholine, adenosine triphosphate, thrombin, serotonin, histamine, and bradykinin acting via endothelial cells realize endothelium-dependent vasodilatation by G protein receptor stimulation that triggers second messenger cascades. Thus, activation of phospholipase C_γ (PLC_γ) induces generation of lipid second messengers inositol trisphosphate (IP_3) and diacylglycerides (DAG), which contribute to an increase in the level of intracellular Ca^{2+} leading to release of the relaxing factor NO synthesized by the endothelium [16, 17]. NO diffuses from the endothelial cells into smooth muscle cells (SMC), activates in them soluble guanylate cyclase, and enhances the cGMP level, which leads to relaxation of smooth muscle tissue via increase in the level of intracellular Ca^{2+} [18].

The effect of ceramide on the endothelium-dependent vasorelaxation exemplifies a pathogenic disturbance of vasorelaxation in hypertension and hypercholesterolemia. Ceramide is produced in endothelial cells upon activation of the sphingomyelin cycle in response to various agents, including oxidized low density lipoproteins (oxLDL), cytokines (tumor necrosis factor-α (TNF-α), interleukin-1β, etc.), growth factors, and also antibodies to CD molecules (CD28, CD40, CD95) [19]. Thus, the basal level of ceramide in endothelial cells of the bovine coronary artery is 72.8 pmol/10^5 cells, or 5.32 nmol/mg protein [20]. In the human thoracic aorta, the amount of ceramide is 307 pmol per mg wet tissue. In atherosclerosis, the ceramide level in the human aorta is 1.5-fold increased to 461 pmol per mg wet tissue [21]. Ceramide can be also detected in a unit endothelial cell of the bovine coronary aorta with immunofluorescent specific antibodies [20]. LDL themselves possess sphingomyelinase activity and can cleave sphingomyelin with production of ceramide. As a result, LDL can be donors of ceramide for vascular cells [22].

Studies on pharmacological effects of synthetic ceramides on isolated small artery perfused under pressure have shown that C_2-ceramide (N-acetylsphingosine) suppresses the bradykinin-induced vasodilation and, thus, is an inducer of endothelial dysfunction in the coronary microcirculation [23]. C_2-Ceramide did not affect the endothelium-independent vasodilation. The term "endothelial dysfunction" is used here and further to denote disorders in the endothelium-dependent vasorelaxation caused by the loss of NO activity in the vascular wall [24]. Direct measurements of the NO level in intact endothelium of the human coronary artery and umbilical vein indicated that the bradykinin-stimulated endothelial concentration of NO decreased in the presence of C_2-ceramide. Therefore, ceramide was concluded to suppress the NO-induced endothelium-dependent relaxation.

Similarly to ceramide, TNF-α-induced generation of O_2^- could underlie the mechanism of decrease in the NO concentration and, as a consequence, disorders in

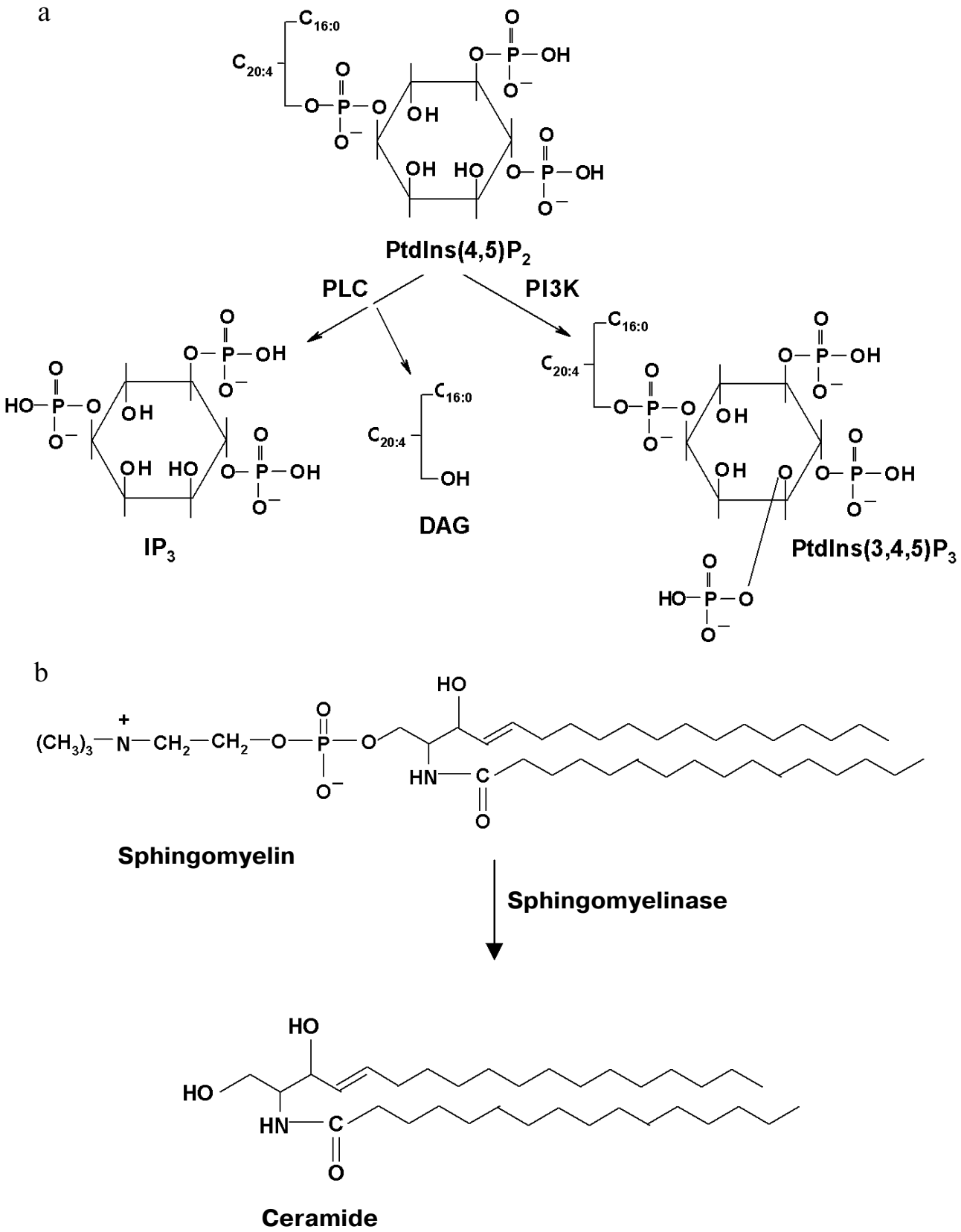

Fig. 1. Structure and generation of lipid second messengers. a) Generation of IP_3, DAG, and PtdIns(3,4,5)P_3 from PtdIns(4,5)P_2. b) Generation of ceramide from sphingomyelin.

the endothelium-dependent relaxation in small coronary arteries [20, 23]. It should be noted that cytokines are released by immunocompetent cells infiltrating the vascular wall in cardiovascular diseases, such as atherosclerosis and myocardial infarction. Not only TNF-α, but also other cytokines affected the endothelium-dependent vasodilation of rat, cat, bovine, and human arteries and veins and, thus, induced pro-atherogenic effects. TNF-α was shown to rapidly activate acid sphingomyelinase in endothelial cells of coronary vessels and increase the level of endogenous ceramide [23].

The further studies of Gulbins and Li [23] indicated that ceramide and sphingosine stimulated $O_2^{\cdot-}$ generation in vascular cells. In fact, treatment of endothelial cells with substances eliminating $O_2^{\cdot-}$ from the extra- and intracellular environment prevented the ceramide-induced endothelial dysfunction and also a decrease in the NO level in bovine small coronary arteries. Ceramide was established to increase the $O_2^{\cdot-}$ level in the small coronary artery endothelium, and this increase could be blocked by different inhibitors of NADPH oxidase. Inhibition of NADPH oxidase prevented the ceramide-induced and $O_2^{\cdot-}$-caused disorder of the endothelium-dependent relaxation of small coronary arteries in response to bradykinin and other agonists. These works have clearly shown that NADPH oxidase mediates the ceramide-induced dysfunction of endothelial cells [23].

In the endothelial cells of coronary vessels, ceramide induced a rapid translocation of $p47^{phox}$ subunit of NADPH oxidase into the cytoplasmic membrane with its subsequent phosphorylation. Ceramide is known to activate atypical protein kinase, e.g. PKC_ζ. Therefore, it must not be ruled out that NADPH oxidase acts through ceramide-dependent phosphorylation of PKC_ζ in response to activation of membrane receptors [23].

However, it was supposed [23] that the major mechanism underlying the effect of ceramide on reactive oxygen species (ROS) production in vascular cells should be associated with the ability of this lipid to combine lipid rafts, microdomains consisting of lipids and membrane proteins, with production of platforms in plasma membranes of endothelial cells [25]. The ceramide-induced production of $O_2^{\cdot-}$ in endothelial cells caused aggregation of NADPH oxidase subunits by integration of lipid rafts containing subunits of this enzyme.

Oxidative stress and the associated endothelial dysfunction contribute to pathogenesis of many cardiovascular diseases, including hypercholesterolemia, atherosclerosis, heart failure, etc. Cytokines influencing vascular wall cells via the sphingomyelin/ceramide cascade are crucial effectors of these processes. Thus, the activation of signal transduction pathways mediated by ceramide and its interaction with the NO cascade is a mechanism of pathogenesis and development of cardiovascular diseases [20, 23, 25].

Vasoconstriction. Endothelin-1 (ET-1) is an important regulator of vascular tone under physiological conditions. ET-1 is a member of the endothelin family synthesized in various cells; it is generated in endothelial cells and is a vasoconstrictive component of the endothelium-dependent regulation of vascular tone [15] (Table 2). ET-1 is produced in endothelial cells under the influence of angiotensin II, vasopressin, thrombin, lipoproteins (LDL and HDL), and insulin. The ET-1 concentration increases upon the interaction of endothelial cells with growth factors: transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), and fibroblast-derived growth factor (FDGF). ET-1 binds with two different G protein-coupled receptors A and B of which the receptor A has a high density on the arterial vasculature. The vasoconstrictor effect of ET-1 is associated with the receptor-dependent activation of certain PLC isoforms, which generates IP_3 and DAG. IP_3 induces constriction of smooth muscle tissue due to its ability to increase the level of intracellular Ca^{2+}. Even 5 min after addition to SMC of the porcine coronary artery, ET-1 causes maximal phosphorylation of myosin light chains (MLC) in the presence of Ca^{2+} under the influence of MLC kinase, and this state of MLC is retained for 60 min [26]. NO lowers the duration

Table 2. Regulatory factors of vascular tone

Factor	Vascular effect	Target	Second messenger	Suppressors	References
NO	endothelium-dependent vasorelaxation	SMC	cGMP (non-lipids)	ceramide, ROS	[15, 19, 22]
Endothelin-1	endothelium-dependent vasoconstriction, vasorelaxation	SMC	IP_3, DAG	NO, prostacyclin	[15, 26]
		endothelial cells	IP_3, DAG		
Angiotensin II	endothelium-independent vasoconstriction	SMC	IP_3, DAG $PtdIns(3,4,5)P_3$	NO, prostacyclin	[4, 27, 31-36]

of the ET-1-mediated vasoconstriction, returning the calcium concentration to the basal level. DAG activates PKC, which mediates the mitogenic effect of ET-1. DAG-activated PKC phosphorylates extracellular signal-regulated kinases (ERK) of the MAPK family, which results in activation of nuclear factors c-fos and c-jun regulating gene expression and mitogenesis [15] (Table 2).

Some data indicate the involvement of DAG in PKC activation and endothelium-dependent vasoconstriction. Thus, stimulation with ET-1 of porcine coronary artery increased the DAG level, which resulted in a steady increase in the PKC activity in the membrane fractions and a decrease in this enzyme activity in the cytosolic fraction. In the presence of ET-1, filament-binding proteins caldesmon and desmin were phosphorylated. This resulted in migration and proliferation of SMC, which were suppressed by PKC inhibitors. And, finally, a PKC activator phorbol ester also induced constriction and phosphorylation of caldesmon and desmin of the smooth muscle of porcine coronary artery [15].

Notwithstanding the vasoconstrictor properties of ET-1, activation of its receptor on endothelial cells leads to a slight vasodilation. The ET-1-induced increase in the intracellular content of Ca^{2+} activates PLA_2 with release of arachidonic acid, which via the cyclooxygenase pathway is metabolized into prostacyclin inducing SMC relaxation through activation of its own receptor on these cells. Moreover, increase in the intracellular Ca^{2+} level in endothelial cells activates endothelial NO synthase (eNOS) and releases NO. Vasodilators NO and prostacyclin suppress the production of ET-1 and its vasoconstrictor and mitogenic effects, i.e. manifest anti-atherogenic properties that have been confirmed in many experiments. The above-described facts exemplify the interaction of different signaling pathways, which are activated by the same agonist (ET-1) [15].

Endothelium-independent vasoconstriction is mediated by the peptide hormone angiotensin II (Table 2), which activates in SMC a spectrum of signaling pathways, and this determines the multiplicity of its functions [27].

The angiotensin II receptor (AT_1) activates PLC through a heteromeric G_q protein. In the vasculature, this receptor is present at high density in arterial SMC and relatively low levels in the adventitia and is undetectable in the endothelium. The signaling cascade produced by the angiotensin II binding with AT_1 provides for immediate events, such as vasoconstriction, and late events, such as cell growth, migration, deposition of extracellular matrix, and inflammation. Angiotensin II promptly activates PLC_β and PLC_γ through G proteins and tyrosine kinases, respectively. PLC_β rapidly (15 sec) produces IP_3, while PLC_γ hydrolyzes phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$) during the later phase. A specific inhibition of AT_1 prevents the angiotensin II-induced hydrolysis of $PtdIns(4,5)P_2$, and this suggests an exclusive role of AT_1 in the stimulation of PLC. IP_3 induces the release of Ca^{2+} from intracellular stores and influx of extracellular Ca^{2+}, whereas DAG activates PKC with involvement of cofactors, phosphatidylserine, and Ca^{2+}. These events occur concurrently with the beginning of constriction of isolated vascular SMC and intact small arteries and present an early signaling pathway triggering calcium-dependent phosphorylation of MLC and cell constriction. The angiotensin II-induced generation of DAG results in translocation of the activated PKC into the plasma membrane where this enzyme phosphorylates the proteins involved in vasoconstriction. This effect is mediated via activation of Na^+/H^+ exchanger leading to intracellular alkalization, important modulator of actin-myosin interaction, and contraction. [27].

In addition to immediate signaling processes associated with vasoconstriction, AT_1 receptor activates multiple signaling pathways responsible for a long-term regulation of the vascular SMC functions, such as migration, formation of extracellular matrix, and synthesis of growth factors. These processes are provoked by cascades triggered by angiotensin II-stimulated signals within a few minutes and include phosphorylation of tyrosine kinases, activation of MAPK, PLA_2, and metabolism of arachidonic acid, etc. Thus, signals of AT1 receptor activate a number of kinases, in particular phosphatidylinositol 3-kinase (PI3K), which phosphorylates phosphoinositides in position 3 of the inositol ring with production of $PtdIns(3,4,5)P_3$, PtdIns(3)P, $PtdIns(3,4)P_2$, and $PtdIns(3,5)P_2$. PI3K influences cell survival and plays an important role in the regulation of vascular SMC growth [28]. In these cells angiotensin II stimulated the generation of $PtdIns(3,4,5)P_3$, inducing translocation of p85 subunit of PI3K from the cytoplasm into the cytoskeleton. This effect of angiotensin II reaches a maximum in 15 min and returns to the control level 30 min later. The involvement of PI3K in the angiotensin II-stimulated hyperplasia of rat SMC in culture is confirmed by prevention of this effect by a specific inhibitor of PI3K. The major molecular target of PI3K is PKB, which modulates the angiotensin II-caused increase in the Ca^{2+} concentration in aortic SMC and protects the cells against apoptosis, influencing Bxl-2 and c-Myc expression and inhibiting caspases [29]. The angiotensin II-activated $PtdIns(3,4,5)P_3$-dependent pathway of signal transduction in vascular cells seems to control the balance between mitogenesis and apoptosis, which are two fundamental processes in the regulation of vascular structure under normal and pathological conditions [4].

Angiotensin II stimulates PLA_2 activity in vascular SMC and endothelial cells with release from membrane phospholipids of arachidonic acid, which is a substrate for cyclooxygenases, lipoxygenases, and cytochrome-450 oxygenase. The PLA_2 activation in response to angiotensin II is mediated by AT_1 receptors, depends on intracellular Ca^{2+}, Ca^{2+}-calmodulin-dependent protein

kinase II, and MAPK, manifests itself some minutes later, and continues for 30 min after the stimulation [27].

Angiotensin II induces a sustained activation of phospholipase D (PLD), which plays an important role in production of second messengers in vascular SMC [30]. Activation of PLD is detected in 2 min, and the enzyme activity is remains elevated for 60 min. In vascular SMC of rabbits, the angiotensin II-induced activation of PLD causes hydrolysis of phosphatidylcholine with production of phosphatidic acid and its subsequent conversion into DAG by phosphohydrolase of phosphatidic acid. DAG induce a long-term activation of PKC. In total, these events form a signaling cascade that provides for a sustained of the angiotensin II-induced activation of PKC in SMC [31].

In rat vascular SMC, βγ-subunits of G protein coupled with the AT_1 receptor is shown to activate PLD by a Src-dependent mechanism [32]. This signaling cascade also includes low molecular weight G proteins (RhoA) [33]. The signal transduction from angiotensin II via PLD activation has been observed in hypertrophy and proliferation of vascular SMC [4, 32-36]. Angiotensin II suppresses interaction of the insulin receptor with PKB/PI3K/PtdIns(3,4,5)P_3, which leads to vascular resistance to insulin [35].

Interaction of Vascular SMC with Extracellular Matrix

Cells of the main blood vessels are constantly exposed to signals from extracellular matrix proteins and intercellular contacts. These signals are very important for maintenance of the cell phenotype and, consequently, cellular functions and also for the cell survival [37-39]. Through transmembrane integrin receptors, the extracellular matrix adhesive glycoproteins fibronectin and laminin bind with components of the vascular cell cytoskeleton and form focal adhesion, which triggers focal adhesion kinase (FAK) and other kinases, as well as phospholipases. Signals (biophysical and biochemical properties of the extracellular matrix, activation of integrins, constricting status of the cytoskeleton, specific features of the extracellular milieu) from integrin clusters are transmitted via systems of second messengers, including PtdIns(4,5)P_2, PLC_γ, tyrosine kinases, Rho family of G proteins, MAPK and many others [40] (Fig. 2). As shown in several cell lines (3T3 cells, human embryonic fibroblasts), phosphorylation on tyrosine residues is the earliest response to integrin binding with extracellular matrix components and other ligands. Phosphorylated tyrosine residues of FAK serve as binding sites for cellular proteins with SH2 and SH3 domains. Phosphorylation of these

Table 3. Role of lipid second messengers in activation of vascular cells by extracellular matrix components

Lipid second messengers	Cell type	Inducers/suppressors	Effect	References
PtdIns(3,4,5)P_3	SMC, 3T3 fibroblasts	Collagen I, collagen IV, fibronectin, laminin, PDGF, wortmannin, LY-294002	Activation/inhibition of proliferation and migration, reorganization of cytoskeleton (actin polymerization)	[42-47]
PtdIns(4,5)P_2*	SMC, endothelial cells	Fibronectin, collagen I, collagen IV, antibodies to α- and β3-integrin complexes, RGD-containing peptide	Formation of focal adhesion Inhibition of SMC migration	[42, 49] [50]
IP_3, DAG	SMC, endothelial cells	Collagen I, collagen IV, fibronectin, laminin, PDGF, acetylated LDL	Proliferation, migration	[45, 52]
PtdIns(3,4,5)P_3 DAG	SMC	Cyclic strain	Enhancement of adhesion to intracellular matrix, maintenance of contractile phenotype, apoptosis	[55-60] [55]

* Precursor of IP_3 and PtdIns(3,4,5)P_3.

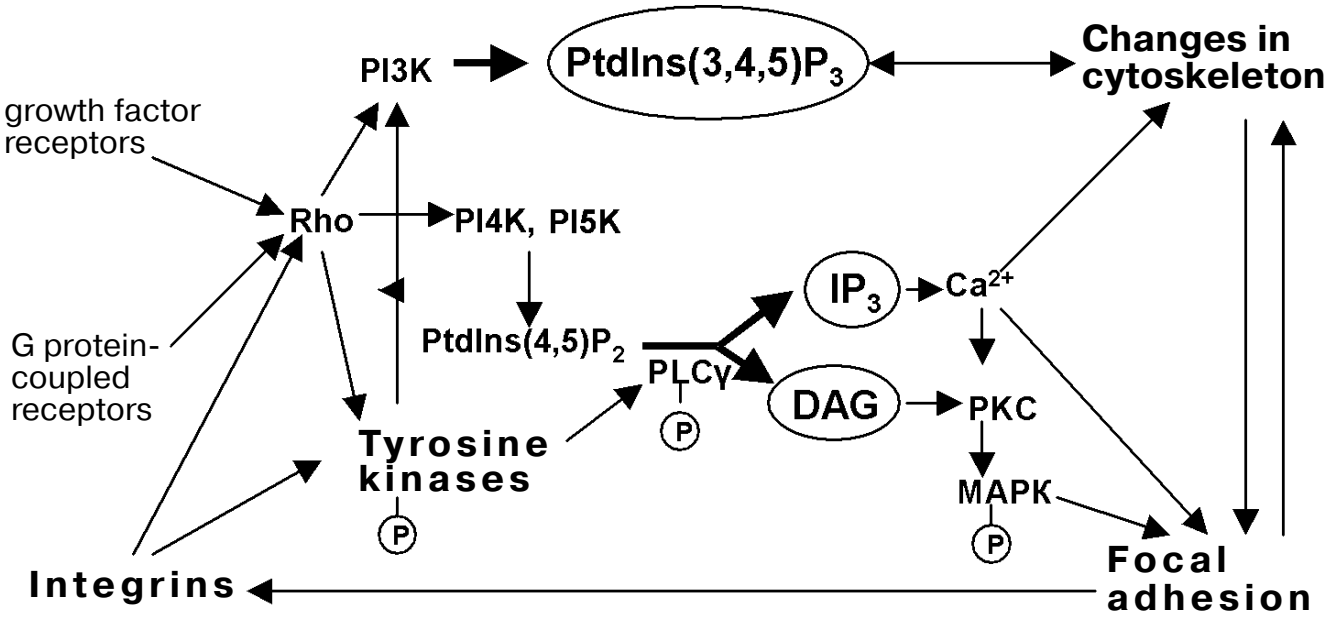

Fig. 2. Involvement of lipid second messengers in focal adhesion.

proteins, including kinases and phospholipases, underlies the transduction of multiple signals from integrins by Src-dependent mechanism [40, 41]. We shall consider only interactions, which occur with generation of lipid second messengers (Table 3 and Fig. 2). Activation of MAPK due to cell adhesion to collagen and/or fibronectin results in phosphorylation of cytosolic PLA_2 ($cPLA_2$), which is involved in cell spreading [42].

PI3K is another kinase activated by binding to phosphorylated tyrosine residues of FAK. This kinase phosphorylates PtdIns(4,5)P_2 with production of PtdIns(3,4,5)P_3. PI3K is associated with focal adhesion and involved in activation of PKB, which induces rearrangement of integrin molecules due to interaction with the cytoskeleton [43, 44]. The importance of PI3K has been shown for the conformational activation of integrins, which enhances their binding with extracellular matrix proteins, and for the cell survival [45-48].

Adhesion of SMC and endothelial cells in culture to fibronectin, I and IV type collagens, and other extracellular matrix proteins was accompanied by increase in the PtdIns(4,5)P_2 level owing to activation of phosphatidylinositol 4- and 5-kinases (PI4K, PI5K) involved in focal adhesion. On the contrary, dissociation of cells from the extracellular matrix rapidly decreased the PtdIns(4,5)P_2 level. Thus, the binding of vascular cells with the extracellular matrix increased the synthesis of PtdIns(4,5)P_2 [49, 50].

Formation of focal adhesion is associated with activation of PLC and releasing of second messengers DAG and IP_3 located in sites of SMC adhesion. The DAG-mediated activation of PKC promotes cell spreading and phosphorylation of FAK, whereas PKC inhibitors prevent production of focal contacts [51, 52]. The transduction pathways of signals from integrins interact with other receptor pathways to enhance or dampen their signals. Tyrosine kinases, growth factor receptors, and integrins act as synergists in the regulation of cell proliferation, adhesion, and migration. In the majority of cell types adhesion to the extracellular matrix is in the stimulation of cell proliferation under the influence of growth factors or serum. It has been noted above that the generation of PtdIns(4,5)P_2 on activation of integrin receptors is an important function of the extracellular matrix providing for reception of signals [42]. Upon PLC cleavage, PtdIns(4,5)P_2 produces DAG that is required for activation of PKC, and IP_3 that induces Ca^{2+} release from intracellular stores. All these processes are closely associated with the proliferative response to growth factors, which is prerequisite for vascular remodeling of injured tissue [52]. These examples show that the regulation of integrin signaling complexes is a molecular bridge between the extracellular matrix and cytoskeleton. In response to mechanical stimuli, focal adhesion components induce cellular responses, such as growth, movement, and differentiation [53].

Effects of Mechanical Stimuli on Vascular Wall

Blood vessels are subjected to stretch and shear stress resulting from blood pressure and blood flow blood pressure, respectively. These mechanical forces are transduced to cells through integrin receptors.

Cyclic stretch. Cyclic stretch in vascular walls appears owing to pulsating pressure generated by the heart. The cytoskeleton perceives tensile signals through integrin receptors which act as transmitters of mechanical forces by rapid remodeling of focal adhesion complexes [54]. The mechanical forces stimulate multiple signaling cascades, such as those of G protein-coupled receptors, receptor tyrosine kinases, and FAK, which transmit the signals through PLC and lipid second messengers IP_3 and DAG [55].

Enhancement of the intracellular Ca^{2+} concentration and PKC activate parallel cascades of signal transduction. Activation of Rac-p38 MAPK leads to apoptosis, whereas activation of ERK induces proliferation of SMC [56, 57]. The balance between these two cascades depends on value and duration of the cyclic stretch. In SMC culture, an excess cyclic stretch triggers signaling pathways, which induce apoptosis [56]. There are data on both increase and decrease in cell proliferation in response to cyclic stress [57]. The cyclic stretch is associated with activation of $cPLA_2$ with the resulting release of arachidonic acid, which is a precursor of eicosanoids, many of which act as vasoconstrictors and can withstand overstrain [58].

Shear stress. Shear stress mainly influences endothelial cells, which are constantly exposed to the blood flow at an angle to the cell surface. The shear stress deforms the cell surface, and this induces local biochemical responses mediated through mechanosensor systems (possible candidates include cytoskeleton/integrins, G proteins, K^+-channels adhesion contact proteins, and caveolas), as well as deformation of the lipid bilayer [58, 59].

Transcriptional and structural changes are observed in endothelial cell culture during several seconds after the initiation of shear stress. As in the case of stretch, these changes are most frequently associated with activation of G protein-coupled receptors and/or receptor tyrosine kinases. These two types of receptors produce IP_3 and DAG upon activation of PLC [60]. The shear stress promptly (in 15 sec) induced a fourfold increase in the IP_3 content in bovine aorta endothelial cells, which remained for 5 min and returned to the basal level within half an hour [61]. Elongation of and alignment of cells are observed concurrently. The inhibitor of phosphatidylinositide metabolism neomycin abolished both effects of the shear stress. Later, in a similar cell model the shear stress was shown to activate the Src-family of receptor tyrosine kinases that could activate PLC [62]. In a culture of ovine fetoplacental artery endothelial cells, a rapid phosphorylation of eNOS was shown, which was caused by activation of PI3K and production of $PtdIns(1,3,5)P_3$ [63]. Shear stress activated ROS generation in endothelial cells, which was followed by release of cytokines and other mediators with production of ceramide, activation of transcription factors, changes in gene and protein expression, cell movement, or cell death [64].

Shear stress also influences the proliferative activity of SMC. Rapid activation of the Rho kinase signaling pathway, delayed production of DAG, and, as a consequence, activation of PKC were observed in culture of human SMC [65]. Many G proteins are activated by shear stress, in particular, G_q protein stimulates PLC activity with production of DAG and IP_3. This pathway of the shear stress signal activates PKC but not other kinases [65].

Interaction of Vascular Wall with Lipoproteins

Interaction of lipoproteins with vascular wall has been studied in a great many works, and specific receptors have been found for intracellular transport of many types of these lipid–protein particles.

Bochkov et al. [66] have recently shown that vascular SMC contain LDL receptors different from classic apo-B,E-(LDL) receptors capable of activating intracellular signaling systems. One of the adhesion molecules, T-cadherin, has been shown to act as a signaling LDL receptor. The binding of LDL and HDL with this receptor activates phosphoinositide- and phosphatidylcholine-specific phospholipases C and D, sphingomyelinases, and PKC, which enhance the level of cytoplasmic Ca^{2+} and activate phosphoinositide metabolism in SMC and endothelial cells in culture [67, 68]. All these cascades are mediated by release of lipid second messengers (IP_3, DAG, ceramide) [69].

As a result of activation of the sphingomyelin/ceramide signaling pathway responsible for the regulation of eNOS activity, LDL decrease the production of NO in endothelial cells [70]. The HDL activity is opposite: they enhance the eNOS activity in endothelial cells through a scavenger receptor, which switches on the signaling pathway mediated through generation of DAG and activation of PKC [71]. LDL induce ROS generation in endothelial cells with involvement of signal-transmitting enzymes, including PLC and $cPLA_2$ [72]. Both LDL and HDL increase the intracellular Ca^{2+} level by activation of signaling pathways via $PLC_\gamma/IP_3/DAG$ [73]. This action of lipoproteins resulted in vasoconstriction. As distinguished from SMC, the activation of PLC with a subsequent increase in the Ca^{2+} level in the endothelial cells led to vasorelaxation because the increase in the cytoplasmic Ca^{2+} level activated the production of NO. HDL is shown to activate Src tyrosine kinase which, in turn, leads to parallel activation of the $PI3K/PtdIns(3,4,5)P_3$/PKB and ERK and their independent activation of eNOS [74]. The above-presented data suggest that the activation of the lipid second messenger systems under the influence of LDL and HDL induces vasoconstriction in SMC and vasorelaxation in endothelial cells. Thus, the ratio of blood concentrations of the two types of lipoproteins controls the vascular tone.

Even in slight hypercholesterolemia, LDL penetrate across the endothelial barrier and are accumulated in vascular wall, where they are subjected to different modifications, including oxidation. OxLDL caused proliferation of rabbit and human aorta SMC via activation of UDP-galactosyl:glucosyl ceramide transferase (Gal-transferase) with production of lactosylceramide [75]. The authors consider lactosylceramide to be a lipid second messenger because it, by contrast with ceramide, stimulates in SMC phosphorylation of p44-MAPK and induces expression of c-fos transcription factor through a specific kinase cascade. All these processes occurred during the first 10 min after the addition of oxLDL into the culture medium. Lactosylceramide also activated NADPH oxidase in SMC of human aorta [75], and the resulting superoxide anion induced cell proliferation activating the same kinase cascade as oxLDL. These data seem to explain the possible role of lactosylceramide in pathogenesis of atherosclerosis.

In culture of human SMC, oxLDL activated components of stress response and apoptotic cell death, such as p38-MAPK and stress-activated protein kinase (SAPK), and also neutral and acidic sphingomyelinases [76]. A specific inhibitor of acidic sphingomyelinase suppressed the effect of oxLDL on these protein kinases. Cell-permeable ceramides mimic the effect of oxLDL. Thus, acidic sphingomyelinase triggers the intracellular signal transduction in SMC after exposure to oxLDL via generation of ceramide by an autocatalytic mechanism.

OxLDL induced different, and sometimes opposite, cellular responses depending on both the LDL oxidation degree and cell type. UV-oxidized LDL displayed a slight mitogenic effect on SMC of the bovine aorta. This effect was mediated through receptor tyrosine kinases, activation of PLC, production of IP_3 and DAG. OxLDL concurrently stimulated neutral sphingomyelinase responsible for hydrolysis of sphingomyelin and increase in the intracellular level of ceramide. The mitogenic effect appeared 30 min after the addition of oxLDL to the cells. OxLDL also activated in SMC acidic and alkaline ceramidases and sphingosine kinase and promoted generation of S1P, which mediated SMC mitogenesis under these conditions [77, 78]. The authors suggested that in vascular cells a ceramide/S1P-rheostat should function and modulate the cell growth and survival in the presence of toxic concentrations of oxLDL.

Chien et al. [79] concluded that PKB activation is a mode of regulation of the oxLDL-caused growth of rat vascular SMC in culture. OxLDL activated the PI3K/PKB signaling cascade triggered by PtdIns(3,4,5)P_3. Suppression of PI3K inhibited the MAPK activation in response to oxLDL. Thus, in culture of rat vascular SMC phosphorylation of p42/p44-MAPK and cell proliferation under the influence of oxLDL are partially caused by the activation of PI3K/PtdIns(3,4,5)P_3/PKB.

Interaction of Vascular Cells with Cytokines and Growth Factors

Atherosclerosis is a chronic inflammatory disease accompanied by recruitment of blood immunocompetent cells into the vascular wall [80, 81]. In the intima milieu, these cells secrete growth factors, cytokines, and other active molecules, which evoke endothelial dysfunction, migration of SMC from the media into the intima milieu, and cell division [82].

Signaling cascades of rat glomerular mesangial cells (which are a model of vascular SMC) can be mimicked by products of the sphingomyelin/ceramide cascade under the influence of cytokines and growth factors [83]. Inflammatory cytokines and growth factors switch on the SAPK and ERK cascade, respectively. Cell-permeable ceramides act similarly to cytokines. On the contrary, sphingosine acts similarly to growth factors. Moreover, ceramide can suppress the ERK cascade activated by growth factors and sphingosine, as well as the cell proliferation. Thus, such sphingolipid metabolites as ceramide and sphingosine can be alternative regulators of cellular processes in atherosclerosis. Later it was found that the activation by ceramide of an atypical kinase PKC_ζ in SMC of rat aorta inhibits the cell growth because of suppression by PKB kinase of cell survival [84].

The mechanism of this inhibition was studied on the culture of muscle cells and vascular SMC exposed to TNF-α, which, concurrently with the PKC_ζ activation, increased the level of ceramide in these cells and induced resistance to insulin. Ceramide-activated PKC_ζ phosphorylates PKB-PH domain on Thr34, and PKB becomes unable to bind with the lipid second messenger PtdIns(3,4,5)P_3, and, as a consequence, the cell survival signal is suppressed. Thus, analysis of interactions of two type lipid messengers, PtdIns(3,4,5)P_3 which activates PKB and ceramide which activates PKC_ζ, is important for understanding how the ERK and SAPK cascades transmitting intracellular signals of proliferation and apoptosis can control mechanisms of cell survival and death. The mechanism of ceramide-stimulated cell death and resistance to insulin is similar to the one described above [85].

On the rabbit carotid artery it was demonstrated that cell-permeable ceramide could diminish SMC proliferation *in vivo* after balloon angioplasty procedure [86]. When the operation was performed using a catheter covered with C_6-ceramide (N-hexanoylsphingosine), the neointimal hyperplasia decreased without noticeable apoptosis. It was supposed that mitogenesis signaling pathways via the PI3K/PKB cascade in response to growth factors should lead to cell survival in a normal vessel and to neointima hyperplasia and restenosis in a vessel upon the balloon angioplasty [87]. In the latter case, cell-permeable ceramide is promising as a therapy for prevention of these undesired processes.

The role of ceramide in apoptosis has been already discussed in some reviews [77, 88-91]. However, the involvement of this lipid in apoptosis of vascular cells is unclear. On the other hand, the PI3K/PKB signaling pathway may be considered as crucial in the regulation of cell survival [26, 27]. Growth factors and attachment of endothelial cells and vascular SMC in culture to extracellular matrix induced the PI3K activation and generation of PtdIns(3,4,5)P_3, which is the major activator of PKB [92-95]. PKB directly influences the ratio of proapoptotic (Bad, Bid, and Bik) and antiapoptotic (Bcl-2 and Bcl-xL) proteins [96-98], phosphorylates caspases on Ser196 with the resulting inactivation of these proteases [99-101], and activates some SAPK which protect the cells against proapoptotic cytokines [102, 103]. Moreover, the PKB-catalyzed phosphorylation of FoxO, NF-κB, and other transcription factors was observed and, as a consequence, some survival genes were activated [104]. As described above, the PI3K/PtdIns(3,4,5)P_3/PKB-initiated cascade interacts with the sphingomyelin/ceramide signaling pathway, and the resulting ceramide inhibits the PKB activation [92].

Thus, the vascular wall is an active, dynamic, and also integrating organ capable of rapidly changing shape and influencing blood flow dynamics. These functions are realized when the vascular wall is exposed to physiological and pathophysiological factors. A small number of second messengers, including lipid metabolites produced upon the binding of these factors with receptors, activate a complicated network of intracellular components transducing external stimuli into physiological responses, such as constriction and relaxation, resistance to mechanical forces of blood flow, protection against thrombogenesis, growth and remodeling, etc. The variety of functional responses mediated by the same second messengers of signal transduction depends on some specific features of unique processes: expression of receptors and ligand–receptor interaction, activation of enzymes followed by release of second messengers and their influences on cytosolic proteins (protein kinases and nuclear factors). Interactions and mutual effects of different signaling pathways essentially determine specific features of the physiological responses. Thus, in some cases, certain signaling cascades can activate other cascades, e.g., by increasing the intracellular level of Ca^{2+} and other ions. On the contrary, the concurrent exposure of the cells to various active ligands can modulate the signal transduction from each of them resulting in an integral physiological response, and just this most often occurs under physiological conditions. The duration of ligand action is very important; thus, late signals of angiotensin II trigger mitogenesis through activation of PLD. Note that atherosclerosis develops in arterial walls as a result of hypercholesterolemia, when via the cascade of molecular events mediated by second messengers including IP_3, DAG, PtdIns(3,4,5)P_3, and ceramide, the vascular wall becomes a scene of such pathophysiological processes as oxidative stress, intracellular accumulation of cholesterol esters, cell migration and proliferation, etc.

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