

Role of Lysophosphatidylcholine (LPC) in Atherosclerosis

Takayuki Matsumoto, Tsuneo Kobayashi and Katsuo Kamata*

Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan

Abstract: Lysophosphatidylcholine (LPC) is a bioactive proinflammatory lipid generated by pathological activities. LPC is also a major phospholipid component of oxidized low-density lipoprotein (Ox-LDL) and is implicated as a critical factor in the atherogenic activity of Ox-LDL. LPC is believed to play an important role in atherosclerosis and inflammatory diseases by altering various functions in a number of cell-types, including endothelial cells, smooth muscle cells, monocytes, macrophages, and T-cells. LPC activates several second messengers -- including protein kinase C, extracellular-signal-regulated kinases, protein tyrosine kinases, and Ca^{2+} -- implicating the engagement of transduction mechanisms in its observed actions. Moreover, recent evidence suggests that in several cell-types, cloned orphan G-protein-coupled receptors may serve as the specific receptors *via* which LPC modulates second messenger pathways (although LPC may not be a direct ligand of such receptors). In addition, current evidence suggests that LPC impairs the endothelium-dependent relaxations mediated by endothelium-derived relaxing factors and directly modulates contractile responses in vascular smooth muscle. However, despite all this, and although elevated levels of LPC have been linked to the cardiovascular complications associated with atherosclerosis, ischemia, and diabetes, the precise pathophysiological roles played by LPC in several states remain to be established. In this review, we focus in some detail on the entirety of the signal-transduction system for LPC, its pathophysiological implications, and the vascular abnormalities associated with it.

Keywords: Atherosclerosis, inflammatory, endothelial dysfunction, LPC, vascular tone, signal transduction.

1. INTRODUCTION

Atherosclerosis, recognized as the main cause of death in industrial countries, is a disease of the blood vessel wall (involving lipid accumulation, chronic inflammation, cell death, and thrombosis) that can lead to heart disease and stroke [1-4]. Although elevated cholesterol levels are a recognized risk factor for atherosclerosis, a growing body of evidence suggests that oxidation of low-density lipoprotein (LDL) is an important, if not obligatory, event in this condition [5-7]. Until recently, oxidized LDL (Ox-LDL) was as-

in vivo [9-11]. Research in this field has been confounded by the complexity of phospholipid biochemistry, the paucity of animal models of human atherosclerotic vascular diseases, and the difficulty of establishing a causal link between specific lipid mediators within the vessel wall and clinical events. Although the clinical and pathological manifestations of atherosclerotic vascular diseases cover a wide range, inflammation is common to all stages of these diseases, and various bioactive lipid mediators are associated with such inflammation in various cells [2-4, 8, 12-18]. The purpose of

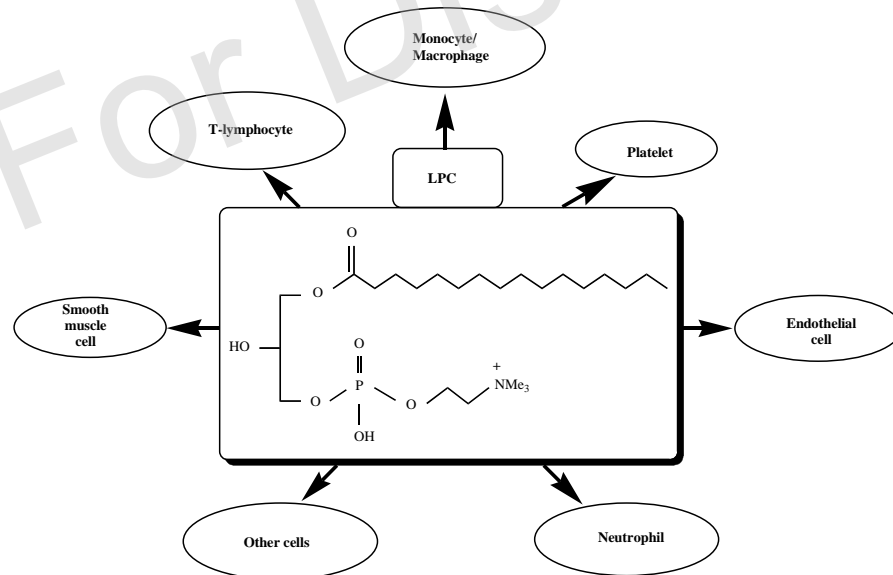


Fig. (1). Cell-types on which LPC is known to have effects.

sumed to yield a final product responsible for several of the reactions involved in atherogenesis [8]. However, the accumulation of several oxidized phospholipids and lysophosphatidylcholine (LPC) has been reported in experimental models of atherosclerosis, raising the prospect of their involvement in proinflammatory processes

this article is to review what is known about LPC, and the inflammatory and vascular abnormalities associated with it.

Metabolism of LPC and its Properties

LPC (1-acyl-sn-glycero-3-phosphocholine, also called lysolecithin) is an important lipid molecule in mammalian tissues (Fig. (1)). Over the past 20 years, abundant evidence has accumulated of direct proinflammatory and atherogenic effects of LPC. Although

*Address correspondence to this author at the Department of Physiology and Morphology, Institute of Medicinal Chemistry, Hoshi University, Shinagawa-ku, Tokyo 142-8501, Japan; Tel/Fax: +81-3-5498-5856; E-mail: kamata@hoshi.ac.jp

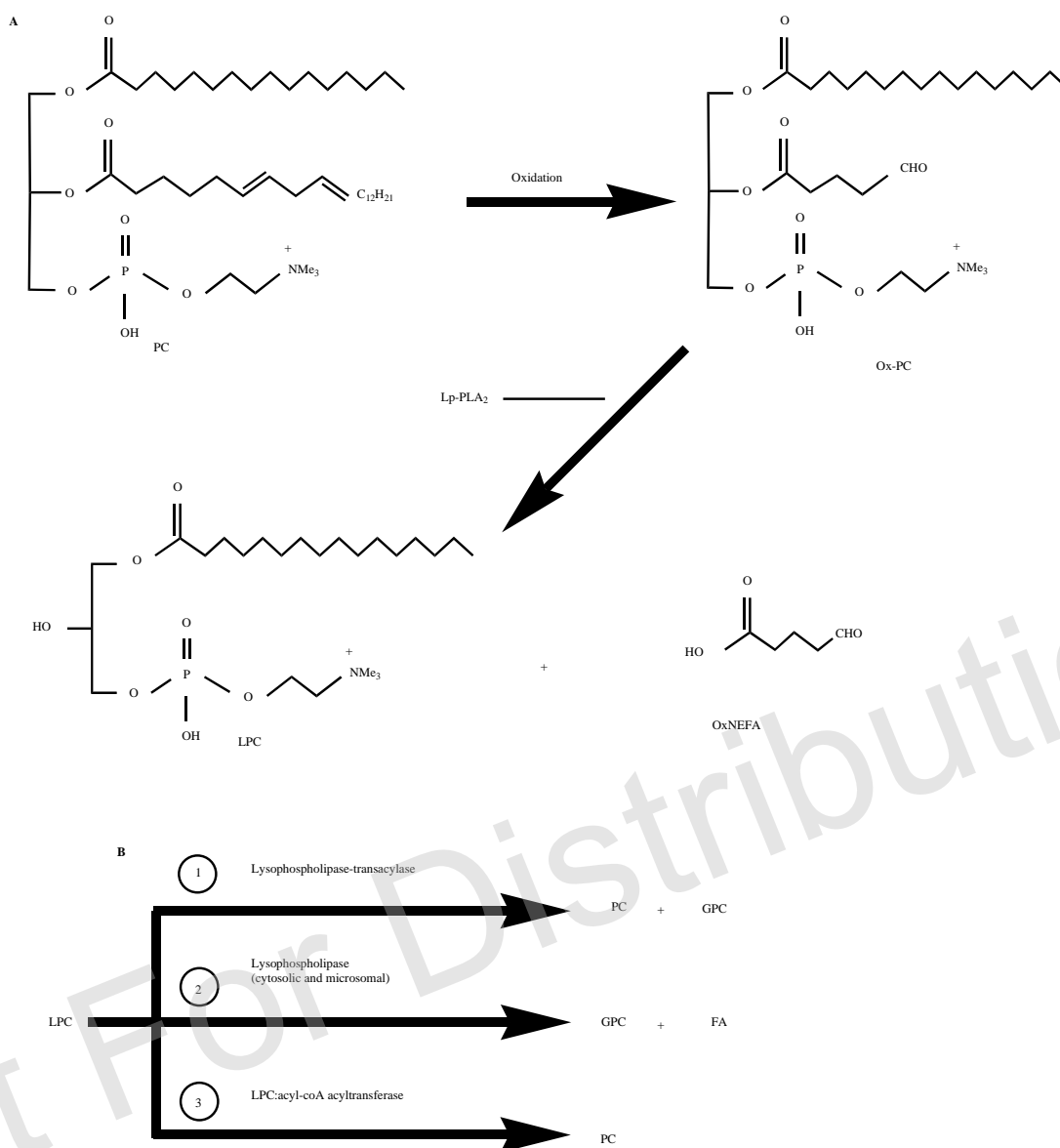


Fig. (2). A: Hydrolysis of oxidized phospholipids by Lp-PLA₂. The increase in LPC content is the result of two sequential events: oxidation and fragmentation of the sn-2 residues of phosphatidylcholine (PC), followed by hydrolysis of the shortened fatty acyl residues by LDL-associated Lp-PLA₂. This results in the generation of two bioactive lipid mediators, LPC and oxidized nonesterified fatty acids (NEFA), which are proposed to play important roles in both the homing of inflammatory cells into lesion-prone areas and local increases in inflammatory mediators. B: Pathways for catabolism of monoacyl LPC. LPC catabolism occurs through a disproportionation reaction involving two LPC molecules catalyzed by cytosolic lysophospholipase-transacylase to form PC and glycerophosphorylcholine (GPC) (*pathway 1*), a hydrolytic pathway catalyzed by lysophospholipase to yield GPC and fatty acid (FA) (*pathway 2*), and a reacylation pathway to form PC, catalyzed by LPC:acyl-CoA acyltransferase (*pathway 3*).

LPC constitutes only 1-5% of the total phosphatidylcholine (PC) content of non-Ox-LDL, as much as 40-50% of the PC contained within the LDL molecule is converted to LPC during LDL oxidation *via* two different pathways [19]. The circulating LPC is generated predominantly by the activity of lecithin-cholesterol acyltransferase (LCAT), which transfers a fatty acid from PC to cholesterol [20, 21]. Higher LCAT activity has been observed in the plasma of atherosclerotic patients with a higher concentration of LPC [22] suggesting that LCAT may be an important factor in the production of LPC in these patients. Moreover, phospholipase A₂ (PLA₂) hydrolyzes PC, simultaneously generating a molecule of LPC and one of arachidonic acid [23, 24].

Recent evidence has established central roles in atherogenesis for two phospholipases: namely, secretory PLA₂ (sPLA₂) [25] and lipoprotein-associated PLA₂ (Lp-PLA₂, alternatively termed platelet

activating factor (PAF)-acetylhydrolase (PAF-AH)) [11,26,27]. sPLA₂ is Ca²⁺-dependent and hydrolyzes the sn-2 acyl group of the glycerophospholipids in lipoproteins and cell membranes to yield LPC and free fatty acids. It is also implicated in isoprostane production from oxidized phospholipids. sPLA₂, an acute-phase reactant that is upregulated by inflammatory cytokines, may represent a new independent risk factor for coronary heart disease. In contrast to sPLA₂, Lp-PLA₂ is Ca²⁺-independent, and it is specific for short acyl groups at the sn-2 position of the phospholipid substrate. Lp-PLA₂ can also hydrolyze oxidized phospholipids to generate LPC and oxidized fatty acids (Fig. (2)). Thus, Lp-PLA₂ plays key roles in the degradation of proinflammatory oxidized phospholipids and in the generation of LPC and oxidized fatty acids. Indeed, circulating Lp-PLA₂ is a marker of inflammation that plays a critical role in atherogenesis, and its inhibition may have anti-atherogenic effects [28, 29]. LPC accumulation reflects increased production *via* PLA₂-

catalyzed PC hydrolysis or decreased LPC catabolism, or *via* a combination of both processes. Catabolism of LPC occurs through three different pathways mediated by separate enzymes [30,31] (Fig. (2)). From numerous reports, there is evidence that the level of LPC in the blood is elevated in a variety of pathophysiological states, such as asthma [32], atherosclerosis [22, 33], diabetes [34-36], hyperlipidemia [19, 37], ischemia [23] and obesity [38].

The physiological concentrations of LPC in body fluids, including blood and ascitic fluid, are very high [39,40]. In addition, LPC may lyse cells at high concentrations due to its detergent-like properties [41]. The LPC molecule is wedge-shaped, consisting of one long hydrophobic fatty acyl chain and one large hydrophilic polar choline headgroup, attached to a glycerol backbone (Fig. (1)). The amphipathic nature of LPC gives it surfactant- and detergent-like properties. At low concentrations, LPC exists as single molecules in solution. However, when its concentration exceeds the critical micelle concentration, LPC can form small micelles, each composed of approximately 180 molecules [42]. Although single LPC molecules can insert readily into the outer layer of the cell membrane, they do not seem to flip quickly into its inner layer [43]. However, micelles formed from LPC may fuse with cell membranes to disturb membrane conformation, and perhaps lyse cells [44], and the detergent effect of LPC is mostly exerted by LPC-produced micelles. Numerous lines of evidence have been accumulated against a simple nonspecific membrane-damaging effect of LPC, and although the transmembrane signal transduction induced by LPC has been studied in many cellular systems it has been suggested that a specific membrane LPC may not exist [45]. In fact, at physiological concentrations if all the LPC were in an active form able to interact with its receptors, the entire population of LPC receptors would be saturated and/or downregulated. A characteristic of LPC is that it is reversibly bound to albumin, erythrocytes, or lipoproteins in the circulation [46-48]. Albumin acts as a reservoir for LPC, effectively controlling LPC bioavailability. Thus, the functionally available concentrations of LPC *in vivo* may be controlled by a balance between different forms of LPC, including the free form, the albumin-bound form, other lipoprotein-bound forms, and/or potentially unidentified forms.

2. INFLAMMATORY AND VASCULAR ABNORMALITIES ASSOCIATED WITH LPC

LPC is believed to play important roles in atherosclerosis and inflammatory diseases by altering various functions in a number of cell-types, including endothelial cells (ECs), smooth muscle cells (SMCs), monocytes, macrophages, and T-cells (Fig. (1), Table 1). Some of the proatherothrombotic effects of lipoproteins have been attributed to the inflammatory effects of LPC, including: a) induction in ECs of adhesion molecules and chemoattractants, b) stimulation of migration and proliferation in vascular SMC (VSMC), c) inhibition of endothelial migration after injury (Fig. (3)), and d) disturbances of vascular tone (Fig. (4)). Recently, LPC has been shown to bind to G-protein-coupled receptors (GPCRs) in lymphocytes and a number of tissues, including the aorta, and thereby to induce receptor internalization, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (MEK/ERK) activation, and chemotaxis. In these ways, LPC can trigger signal-transduction cascades involved in the initiation and development of atherosclerosis. In this section, we review these LPC-induced responses and cellular mechanisms in detail.

2.1. LPC and Inflammatory Responses

Recent research has shown that inflammation plays key roles in coronary artery disease and other manifestations of atherosclerosis. Immune cells dominate early atherosclerotic lesions, their effector molecules accelerate progression of the lesions, and activation of inflammation can elicit acute coronary syndromes [3]. Atherosclerotic lesions (atheromata) are asymmetric focal thickenings of the innermost layer of the artery, the intima [3]. They consist of various cells, connective-tissue elements, lipids, and debris [71]. Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular ECs and SMCs. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium. Most of the cells found in the fatty streak are macrophages, together with some T-cells. In the center of an atheroma, foam cells and extracellular lipid droplets form a core region, and this is bounded by a cap of SMCs and a collagen-rich matrix. T-cells, macrophages, and mast cells infiltrate

Table 1. LPC-Mediated Biological Effects on Putative Inflammatory Cells

TARGET CELLS	EFFECTS	REFERENCES
Neutrophils	ROS generation: NAD(P)H oxidase activation and myeloperoxidase release	[49]
	Functional responses: increased chemotaxis, elastase release	[49]
Monocytes/Macrophages	Formation of inflammatory mediators: upregulation of cytokines (IL-1 β , IL-8, VEGF, HB-EGF), Ca ²⁺ -dependent PLA ₂ enzymes, and arachidonic acid release	[50, 51]
	Functional responses: increased chemotaxis	[52, 53]
	Cytotoxicity: increased cellular permeability and apoptosis	[54]
T-lymphocytes	Formation of inflammatory mediators: upregulation of cytokines (IL-2, IFN- γ , and ROS)	[55, 56]
	Functional responses: increased chemotaxis	[49]
	Cytotoxicity: increased apoptosis	[57]
Endothelial cells	Homing of inflammatory cells: upregulation of adhesion molecules (ICAM-1/VCAM-1), P-selectin, and MCP-1	[58-60]
	Formation of inflammatory mediators: activation of Ca ²⁺ -dependent PLA ₂ enzymes, upregulation of COX-2 and arachidonic acid release	[61]
	Functional responses: impaired proliferation/migration and reduced NO- and EDHF-mediated vasodilation	[19, 62-65]
	Cytotoxicity: increased apoptosis	[66]
Smooth muscle cells	Homing of inflammatory cells: upregulation of MCP-1	[67]
	Oxidative stress: NAD(P)H oxidase activation	[68]
	Functional responses: upregulation of growth factors and increased proliferation and migration	[50, 69]
	Cytotoxicity: increased apoptosis	[70]

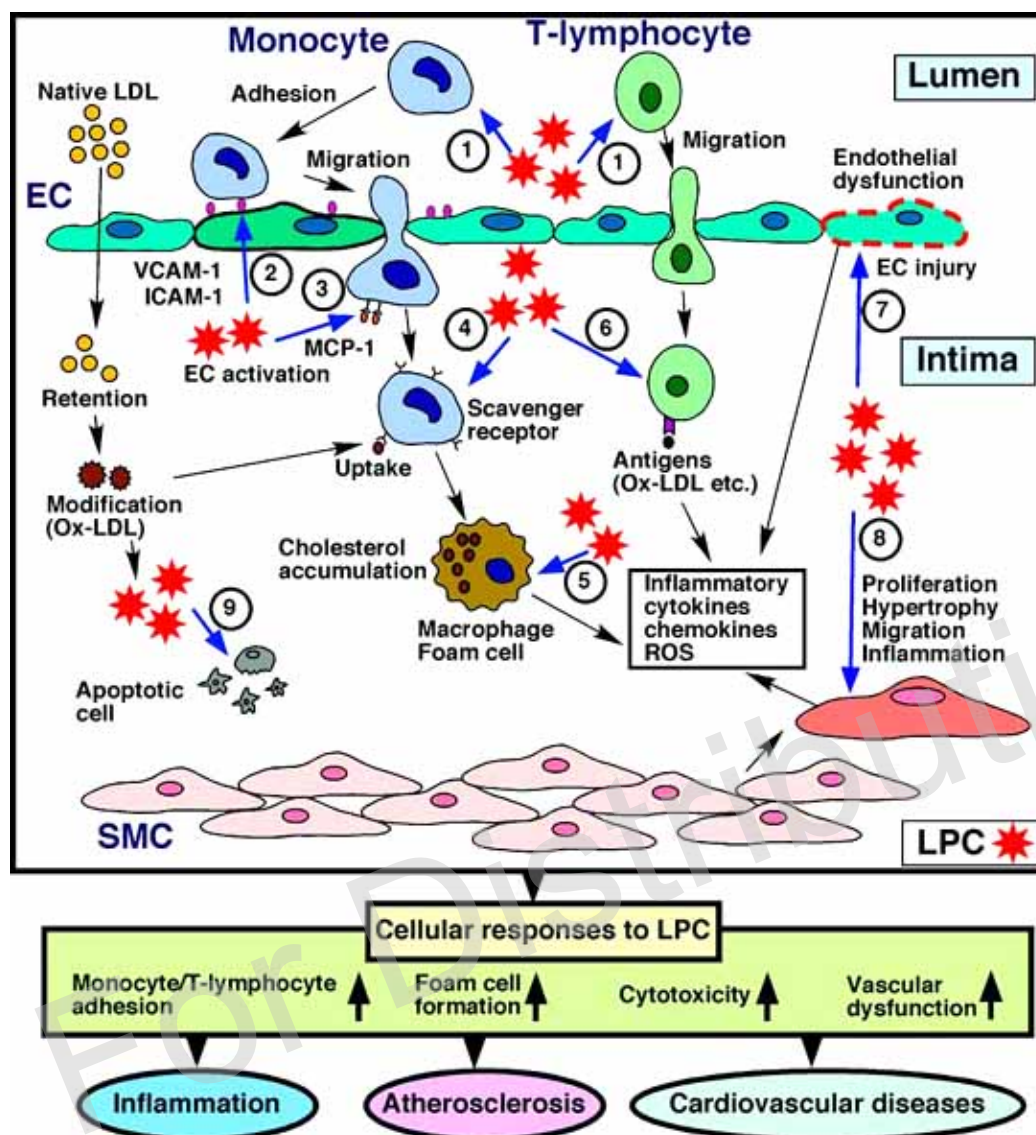


Fig. (3). LPC and atherosclerosis. Native LDL becomes trapped in the subendothelial space, where it can be oxidized by resident vascular cells, such as smooth muscle cells (SMCs), endothelial cells (ECs), and macrophages. Oxidative and enzymatic modifications of LDL lead to the release of inflammatory lipids, including LPC, and these alter the functions and properties of ECs, monocytes/macrophages, T-lymphocytes, and SMCs. The normal arterial EC resists prolonged contact with leukocytes. However, when ECs undergo inflammatory activation, they increase their expression of various leukocyte adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Having adhered to the activated endothelial layer, the monocyte then undergoes diapedesis between intact ECs to penetrate into the tunica intima (innermost layer of the arterial wall). Various chemokines seem to participate in this process, particularly monocyte chemoattractant protein-1 (MCP-1) by interaction, with its receptor CCR2. Once resident within the intima, the monocyte acquires the characteristics of a tissue macrophage. In atheroma in particular, the macrophage expresses scavenger receptors that bind internalized modified lipoprotein particles. These processes give rise to the arterial foam cell, a hallmark of arterial lesions. The foam cell secretes pro-inflammatory cytokines which amplify the local inflammatory response within the lesion as well as reactive oxygen species (ROS). Macrophages may die in this location, some by apoptosis, hence producing the necrotic core of the atherosclerotic lesion. In addition, once resident in the arterial intima a T-cell may encounter antigens such as oxidized-LDL (Ox-LDL), and then produce cytokines that can influence the behavior of other cells present within the atheroma. In the SMC, LPC can induce oxidative stress, adhesion molecules, release of inflammatory cytokines, and chemokines, thereby leading to SMC hypertrophy, migration, and inflammatory responses. Numbering of the different points (1-9) where LPC has effects refers in the text.

the lesion and are particularly abundant in the shoulder region, where the atheroma grows [71]. Many of the immune cells exhibit signs of activation and produce inflammatory cytokines [3].

Accumulation of monocyte-derived foam cells in focal areas of the arterial intima is one of the key events in early atherogenesis. In several animal models of atherosclerosis, localized attachment of circulating monocytes to the arterial endothelium appears to precede the formation of early foam-cell lesions [2, 72]. Although the molecular mechanisms are not completely understood, monocyte

recruitment into these early lesions may involve changes in the endothelial adhesiveness of the monocyte as well as the local generation of soluble vessel wall-derived monocyte chemoattractants, such as monocyte chemoattractant protein (MCP-1) (see below) (Fig. (3)). Activated ECs express several types of leukocyte-adhesion molecules, and these cause blood cells rolling along the

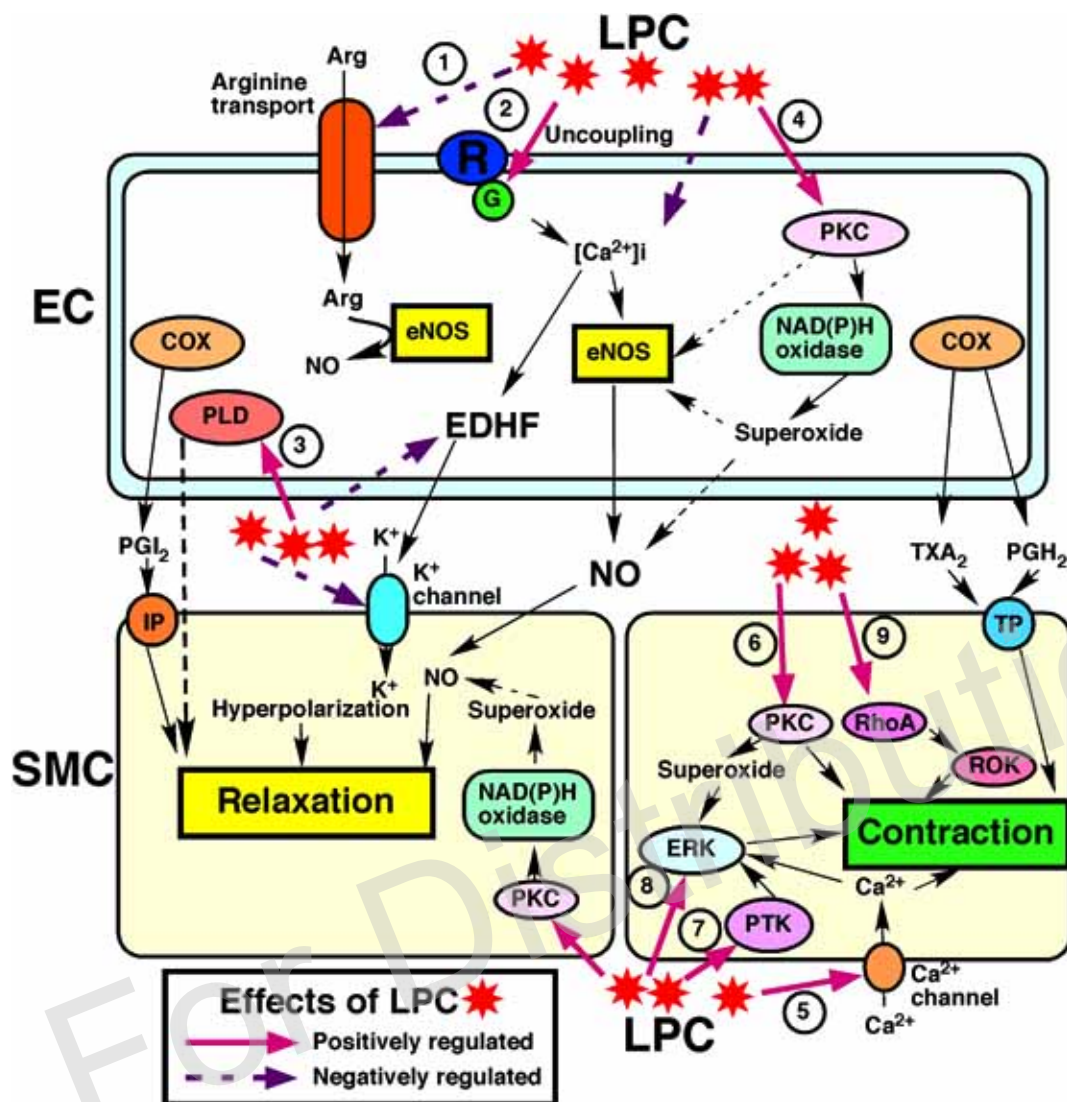


Fig. (4). LPC-induced abnormalities of vascular tone (through both decreased endothelium-dependent relaxation and augmented smooth muscle contraction). Arg, arginine; EC, endothelial cell; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-regulated kinase; G, G-protein; NO, nitric oxide; PKC, protein kinase C; PLD, phospholipase D; PTK, protein tyrosine kinase; R, receptor; ROK, Rho-kinase; SMC, smooth muscle cell. Numbering of the different points (1~9) where LPC has effects refers in the text.

vascular surface to adhere at the site of activation [2, 3, 73]. *In vitro* studies have identified three molecules -- intercellular adhesion molecule-1 (ICAM-1) [74], E-selectin [endothelial-leukocyte adhesion molecule-1 (ELAM-1)] [75], and vascular cell adhesion molecule-1 (VCAM-1) [76] -- on the endothelial surface that are inducible and thereby support the adhesion of various leukocytes, including monocytes [13, 77]. Moreover, P-selectin, a member of the selectin family, is rapidly translocated from platelet α -granules and EC Weibel-Palade bodies to their respective cell surfaces after activation by inflammatory mediators [78]. P-selectin is now known to be largely responsible for the first step in leukocyte-endothelial interaction (i.e., rolling), thus facilitating polymorphonuclear leukocyte activation and adherence [79]. LPC is also a chemotactic factor for human monocytes (point 1 in Fig. (3)) [33]. At non-toxic concentrations, LPC can selectively upregulate the expression of certain inducible endothelial leukocyte-adhesion molecules, in particular VCAM-1 and ICAM-1, in cultured human and rabbit ECs, thus suggesting a potential role in leukocyte recruitment [58] (point 2 in Fig. (3)). Moreover, in the rat mesenteric microvasculature *in vivo*, LPC induces leukocyte rolling and adherence by increasing the surface expressions of P-selectin and ICAM-1, effects that may

be attributable to reduced nitric oxide (NO) release since inhibition of endothelial NO release promotes P-selectin expression [60].

Another important proatherogenic molecule is MCP-1 [16]. MCP-1 is an immediate early gene and is induced by growth factors and inflammatory cytokines [80]. MCP-1, which recruits monocytes (precursors of foam cells) into the arterial wall [72], has been shown to mediate Ox-LDL-induced monocyte chemotaxis in co-cultures of VSMCs and ECs [81]. In mouse models of atherosclerosis, a deficiency of MCP-1 [82, 83] or its receptor, CCR2 [84], has been reported to lead to a ~50-80% reduction in lesion size, while overexpression of MCP-1 has been found to accelerate atherosclerosis progression [85], thereby providing direct evidence of the pathophysiological importance of MCP-1. LPC-induced monocyte chemotaxis could result from the direct chemotactic effect of LPC detected *in vitro* [33]. However, an indirect mechanism involving MCP-1 induction is still a quite important possibility because of the central role that MCP-1 plays in atherogenesis [82, 83, 85], and this may represent a final common pathway for many proatherogenic factors. LPC has been reported to induce an increase in MCP-1 mRNA levels and stimulate the release of MCP-1 protein from human vascular ECs [59, 86] (point 3 in Fig. (3)), and the effect of

LPC on the MCP-1 gene may be mediated through activation of the protein kinase C (PKC) pathway [59]. In addition to ECs and macrophages, VSMCs are another major source of MCP-1 within the vessel wall [67, 87]. In VSMCs, LPC stimulates MCP-1 expression at the transcriptional level [67], and this induction of MCP-1 expression partially involves MEK/ERK, a tyrosine kinase(s), and (to a lesser extent) PKC, but not activation of the platelet derived growth factor (PDGF) receptor [67]. Since LPC is a major component of Ox-LDL (see above), further elucidation of the pathways by which LPC induces MCP-1 production will increase our knowledge of the molecular mechanisms responsible for the potent atherogenic effects of this modified lipoprotein.

Effects of LPC on Monocytes/Macrophages

Monocytes recruited through the activated endothelium differentiate into macrophages (Fig. (3)). A cytokine or growth factor produced in the inflamed intima (namely, macrophage colony-stimulating factor) induces the monocytes entering the plaque to differentiate into macrophages. This step is critical for the development of atherosclerosis [2, 88]. The uptake of modified LDL particles (e.g., Ox-LDL) through scavenger receptor(s) appears to contribute to intracellular lipid accumulation and transformation of these macrophages into foam cells. In the process of foam-cell formation, LPC induces further scavenger-receptor expression on the surface of the macrophage [89] (point 4 in Fig. (3)). In this way, LPC augments foam-cell formation (point 5 in Fig. (3)). Moreover, the macrophages within an atheroma secrete a number of growth factors and cytokines involved in lesion progression and elaboration [4, 88], and they also replicate within the intima [88]. LPC is involved in the production of these factors by macrophages (Table 1). For instance, it stimulates interleukin-1 beta (IL-1 β) production by monocytes in a dose- and time-dependent manner at both protein and mRNA levels [52]. The acyl-chain length of LPC is important for this stimulating effect, no effect being evident when the acyl-chain is less than C16. Saturation is also important, since LPC 18:0 reportedly has a much more powerful effect than LPC 18:1 [52]. IL-1 β , which has been found within human and monkey atherosclerotic lesions [4], is potent at altering endothelial-cell function and regulating VSMC mitogenesis, and it plays a role in the recruitment of leukocytes into the subendothelial space. This may lead to a positive feedback mechanism between LPC and IL-1 β that serves to sustain the inflammatory process.

Effects of LPC on T-Cells

Activated T-lymphocytes are found in early and late atherosclerotic lesions, constituting up to 30% of the cells within the lesion, and they may react to modified LDL. Global T-cell suppression by cytotoxic agents affects the progression of atherosclerosis, whereas the atherosclerotic plaque provides co-stimulatory signals generally required for adequate T-cell stimulation and for the mediation of cellular immune mechanisms [88]. The early and late stages of lesion development are associated with T-lymphocyte infiltration, and with immune activation of both their cytokine production and the humoral response to antigens (Fig. (3)). More than two-thirds of the T-lymphocytes within atherosclerotic plaques have been identified as memory (CD450+) T-cells [90]. Nonactivated CD4 T-cells are also found within such plaques, although their role is unknown. It appears that CD4 T-cell functions may be modulated directly by oxidized epitopes and chemokines. Treatment of anti-CD3-activated CD4 T-cells with LPC has been shown to enhance the expressions of CD40 ligand (CD40L) [91] and cytokine-induced interferon gamma (IFN- γ) [55] *in vitro* in the absence of antigen-presenting cells (point 6 in Fig. (3)). CD4 T-cells express CXCR4, a member of the CXC chemokine-receptor family. Stromal cell-derived factor-1 (SDF-1), an exclusive ligand for CXCR4, is highly expressed in atherosclerotic lesions [73, 92], and is a chemoattractant for CD4 T-cells and a stimulator of chemotaxis *in vitro* [93]. SDF-1 has been shown to enhance both cell-membrane CD40L

expression and the production of the inflammatory cytokines IFN- γ and IL-12 in anti-CD3-activated CD4 T-cells [94]. In human CD4 T-cells, LPC upregulates CXCR4 [56], an effect that can be suppressed by inhibition of nuclear factor- κ B (NF- κ B) signaling or by suppression of the expression of G2A, the putative receptor for LPC (see below). Moreover, LPC enhances the CD4 T-cell chemotaxis seen in response to SDF-1, as well as the SDF-1-stimulated production of the inflammatory cytokines IL-2 and IFN- γ [56]. Thus, the presence of LPC and SDF-1 within atherosclerotic lesions may trigger inflammatory responses mediated by CD4 cells, and these may play an important role in the progression of atherosclerosis.

Effects of LPC on ECs

Atherosclerosis is associated with alterations in a variety of endothelial functions [2, 5, 8, 40, 88]. The pro-inflammatory effects of LPC in ECs have been widely described (point 7 in Fig. (3)) (Table 1). In fact, LPC can induce expressions of ICAM-1, VCAM-1, P-selectin, plasminogen activator inhibitor-1, MCP-1, cyclooxygenase-2 (COX-2), endothelial nitric oxide synthase (eNOS), and growth factors in ECs (Table 1) [95-101]. COX-2, one of two isoforms that catalyze the formation of prostaglandins from arachidonic acid, has been detected in macrophages, SMCs, and ECs within human atherosclerotic lesions [102]. Several studies have also found that COX-2 is involved in the destabilization of atherosclerotic plaques, leading to rupture and atherothrombotic syndromes [102]. Induction of COX-2 expression leads to the extracellular secretion of prostanoids, which then stimulate cell growth [103, 104].

eNOS plays a crucial role in blood-pressure homeostasis and maintaining in vascular integrity [16, 105-107]. LPC has been shown to stimulate the EC production of reactive oxygen species (ROS) [108, 109] and to increase eNOS expression *via* enhanced transcription [96, 101, 110]. Deletion analysis of a human eNOS promoter-luciferase construct led to Sp1 sites at -104 to -90 and PEA3 sites at -40 to -24 being identified as factors in LPC-induced promoter activity [110]. Gel-shift assays have revealed that LPC augments Sp1-binding activity [110]. Subsequent analysis of the signaling events involved in the stimulatory effects of LPC on promoter activity revealed a PI-3K γ -related pathway [111]. In this pathway, PI-3K γ activates JAK2, which in turn activates MEK1. MEK1 then stimulates the binding of Sp1 to the eNOS promoter through activation of ERK1/2, and subsequently increases protein phosphatase 2A activity [110, 111]. During the last few years, it has become clear that ROS produced in mammalian cells can serve physiological roles as signaling molecules, although when produced in excess they can participate in the initiation of disease. Increased expression of eNOS may represent a compensatory mechanism to preserve biological levels of NO in the face of increased ROS production. This compensatory mechanism may explain the observations of increased eNOS levels and NO production in certain pathological states, such as hypertension [112], atherosclerosis [113], and some models of diabetes [114].

Conceivably, LPC could induce gene transcription for a great variety of molecules expressed in the endothelium of atherosclerotic arteries. For example, LPC activates PKC [115, 116], which leads to expression of ICAM-1 in isolated porcine coronary arterial endothelium [117]. The promoter regions of the LPC-inducible genes in ECs have several binding sites for transcription factors such as NF- κ B and activator protein-1 (AP-1), and the activities of NF- κ B and AP-1 are known to be in part modulated by the PKC signal-transduction system [118, 119]. Thus, LPC could regulate the DNA-binding activities of these transcription factors through the mechanism(s) involved in a PKC-mediated pathway, leading to the induction of a number of the endothelial genes implicated in atherosclerosis. Indeed, LPC can induce biphasic regulation of NF- κ B activity in human vascular ECs, and this effect is partly mediated through a PKC-dependent pathway [120]. LPC could play an

important role in the pathogenesis of atherosclerosis by modulating the expressions of some endothelial genes through a regulation of the activation of the transcription factors NF- κ B and AP-1 in atherosclerotic arterial walls.

Effects of LPC on VSMCs

Transformation of VSMCs from a contractile phenotype to a proliferative/secretory phenotype is a hallmark of atherogenesis [121]. Vascular-wall remodeling, manifest as increases in proliferation and apoptosis, is an important step -- in combination with an increase in the synthesis and release of inflammatory cytokines -- in the generation and maintenance of the atherosclerotic plaque [13, 16]. LPC stimulates the productions of IL-6, IL-8, and granulocyte-macrophage colony stimulating factor (GM-CSF) in human coronary artery SMCs [122]. The IL-6 secreted from VSMCs makes an important contribution to inflammation, in part by inducing B-cell differentiation, T-cell activation, and the synthesis of such acute-phase proteins as C-reactive protein (CRP) and fibrinogen in the liver [16]. IL-6 also increases SMC proliferation in a PDGF-dependent manner [16]. IL-8, which is predominantly released by macrophages and ECs, has multiple effects, including neutrophil chemotaxis, activation of 5-lipoxygenase, and the release of cell matrix-resorbing gelatinase and elastase. Stimulation of IL-6 and IL-8 release by LPC in SMCs could represent a pleiotropic role for LPC in atherogenesis, by stimulating both inflammatory (e.g., chemotaxis and lipoxygenase activation) and remodeling pathways (e.g., proliferation and matrix protease activity) in multiple cell-types within the vascular wall. The proliferative effect of LPC in SMCs is also due to the autocrine actions of basic fibroblast growth factor-2 (FGF-2) upon its release in response to LPC stimulation [123]. This raises an interesting potential role for LPC as a mediator of vascular-wall remodeling in atherosclerosis, involving stimulation of both inflammation and cell proliferation (point 8 in Fig. (3)).

LPC and Apoptosis

The development of the atherosclerotic plaque depends upon the balance between the mitosis and death of both the resident cells and those recruited into the lesion. Over the past few years, a role for programmed cell death in the pathogenesis of atherosclerosis has been clearly demonstrated. Disruption of the normal apoptotic process may lead to immune dysregulation, chronic local inflammatory responses, and/or rupture of complex atherosclerotic plaques [124]. It has recently been discovered that LPC functions as a major mediator in both inflammation and apoptotic cell death, with recognized effects on multiple immune cell-types (point 9 in Fig. (3)). The leading role of LPC as an attraction signal in the process of programmed cell death has been shown by Lauber *et al.* [125], who found that the disposal of apoptotic cells is initiated by the secretion of LPC as a chemotactic signal that induces the recruitment of phagocytes. Moreover, Zurgil *et al.* [57] suggested that LPC triggers apoptosis in activated peripheral blood lymphocytes, in association with an increased generation of reactive oxygen species (ROS) and an increase in the Bax/Bcl-2 ratio. Actually, LPC induces apoptotic cell death not only of lymphocytes, but also of other cells (Table 1) (point 9 in Fig. (3)). Treatment of ECs with LPC stimulates several intracellular signaling events: activation of caspase-3, Ca²⁺ influx, phosphorylation of MAP kinases, and production of ROS, all of which can lead to apoptosis [126]. Moreover, a novel interaction between LPC and pro-apoptotic Bid protein has been shown to prime mitochondria for the release of apoptogenic factors [127].

All the evidence -- including the reported effects of LPC on such cells as ECs, VSMCs, monocytes/macrophages, and T-cells -- implies that LPC may play an active role in atherogenesis, and moreover that the selective inhibition of LPC could represent a novel strategy for the inhibition of the progression of atherosclerosis.

2.2. LPC and Vascular Tone

LPC and Endothelial Dysfunction

Several lines of evidence suggest that endothelial dysfunction plays a key role in the development of both macro- and microangiopathy in patients with such inflammatory-associated diseases as atherosclerosis, hypercholesterolemia, and diabetes, and in animal models of these diseases [128-141]. The endothelium controls vascular smooth muscle tone through the production of vasodilator mediators such as NO, prostacyclin (PGI₂), and a still-elusive endothelium-derived hyperpolarizing factor (EDHF) [135, 138, 142, 143]. Current evidence suggests that LPC inhibits endothelium-dependent relaxations (EDRs) mediated by endothelium-derived relaxing factors, and it is likely that LPC causes vascular spasm [65, 115, 117, 144, 145] (Fig. (4)). Indeed, we and others have demonstrated that the EDR induced by ACh is greatly attenuated by pretreatment with LPC [65, 145, 146]. Some years ago, Kugiyama *et al.* [115] found that LPC inhibited thrombin-induced inositol triphosphate (IP₃) production and attenuated [Ca²⁺]_i elevations in human umbilical vein endothelial cells (HUVECs), and they also found that thrombin-induced EDR in aorta was inhibited by LPC. In addition, Murohara *et al.* [147] reported that LPC elicited a further contraction on top of the plateau contraction evoked by prostaglandin F_{2 α} in the pig coronary artery; they suggested that this additional contraction was caused by LPC-mediated inhibition of endothelium-derived NO release. Furthermore, Cowan and Steffen [148] found that in rabbit abdominal aorta, LPC inhibits the EDRs mediated by NO and EDHF in a manner independent of PKC activation. Fukao *et al.* [64] noted that in the rat mesenteric artery, LPC specifically inhibits the hyperpolarization and relaxation due to EDHF (versus the relaxation due to endothelium-derived NO). Although these results suggested that LPC might cause endothelial dysfunction *via* a reduced production of and/or impaired signaling by endothelium-derived relaxing factors (i.e., NO and EDHF), several reports have suggested that the LPC-mediated impairment of EDR can be attributed to interventions in several signaling pathways (Fig. (4)). For instance, LPC impairs high-affinity arginine transport in ECs, thereby disrupting arginine uptake [149] (point 1 in Fig. (4)) and also blocks G-protein-dependent signal-transduction pathways by selective uncoupling of the receptor from the G-protein [150] (point 2 in Fig. (4)). LPC has been found to stimulate phospholipase D (PLD) activity in human cultured EC [151] (point 3 in Fig. (4)), while in isolated blood vessels a close association has been documented between the ability of LPC (and other mediators) to stimulate vascular PLD activity and inhibit EDR [152]. LPC may also affect endothelial function by stimulating the endothelial PKC pathway [153] (point 4 in Fig. (4)), which causes sustained vasoconstriction upon stimulation by agonists such as phenylephrine, angiotensin, and thrombin. PKC is also associated with increased superoxide production [154, 155], which may interfere with NO bioavailability. Indeed, LPC can activate endothelial NAD(P)H oxidase, thereby enhancing superoxide production [155]. Future studies will be required to elucidate the mechanism underlying LPC-induced endothelial dysfunction.

LPC and Vascular Smooth Muscle Tone

It is known that LPC acts directly on vascular smooth muscle to affect vascular tone. Exposure to LPC causes Ca²⁺ influx in cultured VSMC [156-159] (point 5 in Fig. (4)). It has also been reported that in the rat permeabilized small mesenteric artery, LPC increases Ca²⁺ sensitivity *via* PKC activation [160] (point 6 in Fig. (4)), while in the rat isolated perfused mesenteric arterial bed, LPC potentiates phenylephrine responses through a modulation of thromboxane A₂ [161]. In addition, we found in the rat endothelium-denuded aorta that while LPC did not itself produce contraction, it potentiated the vascular contractile responses induced by high-K⁺, UK14,304 (5'-bromo-6-[2-imidazolin-2-yl-amino]-

quinoxaline) (a selective α_2 -adrenoceptor agonist), and phorbol ester [162]. Since LPC markedly potentiated the UK14,304-induced contraction, and the UK14,304-induced response was strongly inhibited by PKC inhibitors [162], it would seem that LPC potentiates the elevation of $[Ca^{2+}]_i$ induced by PKC activation in the rat aorta, and it may selectively potentiate the α -adrenoceptor-stimulated contraction mediated by PKC activation. In that study, LPC was still able to potentiate the high K^+ -induced contraction in PKC inhibitor-treated tissues, suggesting that PKC-independent mechanisms are also involved in the enhancing effect of LPC on smooth muscle contraction in the rat aorta.

It is well known that the protein tyrosine kinase (PTK) and ERK (a subfamily of MAPK) pathways are implicated in a wide range of cellular functions, including cell growth, differentiation, proliferation, and vascular contraction [163-166]. It has been shown that LPC has a mitogenic effect on VSMCs [156, 167, 168] and that activation of membrane or cytoplasmic PTKs may play a key role in LPC-induced mitogenic-signaling responses [169]. Moreover, Ozaki *et al.* [170] reported that in bovine aortic ECs, LPC activates the ERK- and *c-jun* N-terminal kinase (JNK)-MAPK cascade *via* a PTK-dependent pathway. Indeed, several studies have demonstrated that in a number of vessels, α_2 -adrenoceptor-mediated vasoconstriction occurs through activation of ERK2 [165, 170]. Our previous studies [171, 172] demonstrated that LPC potentiates contractile responses in the endothelium-denuded aorta *via* activation of PTKs and/or ERK, which in turn regulates Ca^{2+} influx, and we reported elsewhere that PD98059 (an inhibitor of ERK) markedly attenuated the contraction and ERK2 activity induced by UK14,304 [173] (points 7 & 8 in Fig. (4)). Moreover, the potentiating effects of LPC on UK14,304-induced contraction and ERK2 activity were also inhibited by PD98059. These results suggest that both the UK14,304-induced contraction and its modulation by LPC may be regulated *via* the ERK pathway.

It has become apparent in recent years that the effect of constrictor agonists may be explained not only by an elevation of $[Ca^{2+}]_i$, but also by a decrease in the activity of smooth muscle myosin phosphatase (SMMP), with these two events leading ultimately to an increased phosphorylation of the myosin light chain [174]. Although the regulation of SMMP is not yet completely understood, SMMP has been identified as a target for Rho-kinase, which is regulated by the small GTP-binding protein, RhoA [174], and stimulation of this signaling cascade (involving RhoA, Rho-kinase, and SMMP) has been termed Ca^{2+} -sensitization [174]. Ca^{2+} -sensitization seems to be not only of major importance for the regulation of vascular tone, but to be critically involved in the development of vasospasm in large and small arteries [175, 176]. It has been demonstrated that mildly oxidized LDL can stimulate the RhoA/Rho-kinase pathway in ECs [177]. Moreover, Galle *et al.* [178] reported that LPC and/or OxLDL stimulate the RhoA pathway, resulting in a potentiation of angiotensin II-induced vasoconstriction (point 9 in Fig. (4)).

Yeon *et al.* [179] observed that in rabbit coronary SMCs, LPC decreased the delayed rectifier K^+ current (I_{dk}), and that a PKC inhibitor suppressed the LPC-induced inhibition of I_{dk} . There is other evidence to suggest that PKC inhibits I_{dk} [180], which determines the resting membrane potential in the rabbit coronary SMCs [181], and that depolarization of the membrane potential by inhibition of I_{dk} increases $[Ca^{2+}]_i$ by opening voltage-dependent Ca^{2+} channels, leading to an increase in vascular tone [182]. Hence, an LPC-induced inhibition of I_{dk} may be, at least in part, responsible for the abnormal vascular reactivity seen in the atherosclerotic coronary artery. Taken together, the above data suggests that LPC induces a dysregulation of vascular tone through the effects of several molecules in ECs and VSMCs.

2.3. LPC and Signal Transduction

As mentioned above, both the biological effects and the signaling properties of LPC have been studied *in vitro* in cells that are important in atherosclerosis, including ECs, SMCs, monocytes, and lymphocytes (Table 1). LPC can activate several second messengers -- including PKC, PLC, PLD, ERKs, PTKs, and Ca^{2+} [39, 62, 158, 169, 170, 183] -- implicating the engagement of transduction mechanisms in the observed effects of LPC. Moreover, LPC activates the redox-sensitive transcription factors NF- κ B [120] and AP-1 [118] through the MAPK and PKC pathways. LPC is also known to enhance cAMP response element-binding protein (CREB)/activating transcription factor activity in ECs [184]. As mentioned above, LPC has been demonstrated to be atherogenic, but certain anti-atherogenic actions of LPC have also been reported [45, 185]. It will be important to determine whether this dual function of LPC is related to a) receptor-mediated vs. receptor-independent effects, or b) effects of LPC mediated *via* different types of proteins/receptors.

A few years ago, orphan GPCRs were identified as receptors for LPC, and it was reported that LPC binds to G2A and GPR4, two Gi-protein-coupled receptors, and thereby regulates both cell growth and immunologic responses [186-188; however, see below]. Rikitake *et al.* [189] demonstrated that G2A is abundantly expressed in monocytes/macrophages, and stimulation of macrophage and T-cell chemotaxis by LPC is mediated by G2A [190-192]. This might suggest that G2A promotes monocyte or T-cell recruitment into the arterial wall during atherogenesis. Further, Parks *et al.* found that in $G2A^{-/-}$ $LDLR^{-/-}$ mice, intimal macrophage accumulation at lesion-prone sites in the aorta was reduced in the absence of any detectable effect on T-cell recruitment, that and circulating high-density lipoprotein (HDL) cholesterol was increased after extended periods of Western-diet intervention. Thus, although G2A seems to provide a pro-atherogenic stimulus *in vivo* that is consistent with its chemotactic action, a pleiotropy of effects, including modulation of lipoprotein metabolism, may also contribute to its total effect [193,194]. On the other hand, Hedrick and colleagues demonstrated in mice that an absence of G2A promoted monocyte/endothelial interactions in the aorta [195], and that endothelial G2A expression may aid in the prevention of vascular inflammation and atherosclerosis.

Concerning GPR4, Lum *et al.* [196] found that while GPR4 mRNA was expressed in human vascular ECs, G2A was not. They therefore suggested that in ECs, GPR4 may be a potential GPCR by which LPC signals proinflammatory activities. Moreover, Qiao *et al.* [197] demonstrated that ECs infected with a retrovirus containing a small interference RNA (siRNA) affecting GPR4 exhibit ~50% less GPR4 expression, with a corresponding partial prevention of the following LPC-mediated effects: 1) decrease in transendothelial resistance, 2) stress-fiber formation, and 3) activation of RhoA. They suggested that the dysfunction of the endothelial barrier induced by LPC was regulated by the endogenous GPR4 within ECs, and suggested that GPR4 may play an important role in the inflammatory responses activated by LPC. Huang *et al.* [198] found that LPC, but not low pH, increased monocyte transmigration and RhoA activation, and that these LPC-mediated responses could be blocked by reducing GPR4 expression.

However, shortly after their first description as lipid receptors [186, 187], crucial binding data could apparently not be reproduced, thus leading to the retraction of papers [186, 187] concerning GPR4 and G2A. Although no direct action of LPC towards these receptors has been demonstrated, it is possible that activation of G2A and GPR4 is involved in some way(s) in the complex mechanisms underlying the cardiovascular effects of LPC in several pathophysiological states. Although the physiological functions and endogenous expressions of putative LPC receptors, as well as their patho-

logical implications for cardiovascular diseases, have yet to be fully elucidated, these receptors do seem to be associated in some way(s) with cardiovascular diseases. Future studies will be required for the identification of specific LPC receptor(s) and to establish the relevance of their signaling to pathophysiological states.

Reports have been made of transactivation of receptor tyrosine kinase (RTK) in response to stimulation of a number of GPCRs (e.g., transactivation of the epithelial growth factor receptor by GPCR ligands such as thrombin, angiotensin II, lysophosphatidic acid, and endothelin-1) [199, 200]. That LPC plays a role in the pathogenesis of atherosclerosis and systemic disease is well documented [201], even though a putative specific cell-surface receptor for LPC was not identified until recently [156]. Although it still remains to be clarified which GPCRs are responsible for the action of LPC, Fujita *et al.* [202] recently found that LPC caused Flk-1/KDR transactivation in HUVECs. Since LPC has been implicated in pathological states in vascular ECs that may be related to the progressive pathological events of atherosclerosis, and since Flk-1/KDR activation is an important factor in atherogenesis [203], LPC-induced Flk-1/KDR transactivation could well be a step in the pathological development of atherosclerosis.

The mechanisms by which LPC acts on several target molecules have not been completely elucidated. LPC is an amphiphile possessing a charged headgroup, like phospholipids, but it has only a single aliphatic hydrocarbon chain as a consequence of the hydrolytic cleavage of one of the two aliphatic hydrocarbon groups of phosphatidylcholine [204]. As an intermediate in the metabolism of phosphatidylcholine, LPC is present in a variety of mammalian tissues [205], and it has several theoretical sites of action in cells. For example, LPC can readily incorporate into the sarcolemma, and incorporation of LPC into the membrane phospholipid bilayer results in a significant perturbation of the orderly packing of phospholipid molecules and an alteration of the normal conformation of integral membrane proteins, such as ion-channels [206]. Moreover, LPC may interact directly with target molecules such as ion-channel proteins. LPC has easy access to both the intracellular and extracellular sides of the membrane, and it may bind to channel proteins so as to alter the conductance of the channels. Indeed, a recent report suggested that LPC directly activates the TRPC5 calcium channel [207]. Moreover, LPC may indirectly modulate molecules through secondary intermediates, such as superoxide anions [108, 154]. Future detailed studies of the chemical requirements of such effects will resolve these issues.

The above findings highlight the possible biological role of LPC and provide new molecular insights into the activation mechanisms by which LPC may affect cellular signaling pathways. Future research into the roles played by vascular signaling mechanisms in the vascular pathology of atherosclerosis and associated diseases should continue to focus on physiological and therapeutic means of inhibiting the LPC signal-transduction cascade.

3. CONCLUSIONS

There is now considerable evidence for a role of LPC in atherogenesis. *In vitro* studies have demonstrated that LPC has effects on vascular wall cells that could contribute to all stages of atherosclerosis. Determination of the pathophysiological functions of LPC and its receptors is of primary importance, particularly since they are likely to be involved in many important biological systems and processes, such as the cardiovascular system, immunology, and inflammation. The signaling mechanisms (receptor-mediated and/or receptor-independent signal transduction of LPC) have not yet been studied extensively. However, a better understanding of the regulation of LPC signaling, for example, may provide new insights into the mechanisms responsible for cardiovascular diseases and ultimately lead to novel therapeutic strategies with the potential to improve prognosis.

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ABBREVIATIONS

AP-1	=	Activator protein-1
CCR2	=	Chemokine receptor 2
CD	=	Cluster of differentiation number
CD40L	=	CD40 ligand
COX-2	=	Cyclooxygenase-2
CREB	=	cAMP response element-binding protein
CRP	=	C-reactive protein
CXCR	=	Chemokine receptor
ECs	=	Endothelial cells
EDHF	=	Endothelium-derived hyperpolarizing factor
EDRs	=	Endothelium-dependent relaxations
eNOS	=	Endothelial nitric oxide synthase
ELAM-1	=	Endothelial-leukocyte adhesion molecule-1
ERK	=	Extracellular signal-regulated kinase
FGF-2	=	Fibroblast growth factor-2
G2A	=	G2 accumulation
GM-CSF	=	Granulocyte-macrophage colony stimulating factor
GPCRs	=	G-protein-coupled receptors
HDL	=	High-density lipoprotein
HUVECs	=	Human umbilical vein endothelial cells
ICAM-1	=	Intercellular adhesion molecule-1
I_{dk}	=	Delayed rectifier K^+ current
IFN- γ	=	Interferon gamma
IL-1 β	=	Interleukin-1 beta
IP ₃	=	Inositol triphosphate
JNK	=	<i>c-jun</i> N-terminal kinase
LCAT	=	Lecithin-cholesterol acyltransferase
LDL	=	Low density lipoprotein
LDLR	=	LDL receptor
LPC	=	Lysophosphatidylcholine
Lp-PL A ₂	=	Lipoprotein-associated PLA ₂
MAPK	=	Mitogen-activated protein kinase
MCP-1	=	Monocyte chemoattractant protein
NF- κ B	=	Nuclear factor- κ B
NO	=	Nitric oxide
Ox-LDL	=	Oxidized LDL
PAF	=	Platelet activating factor
PAF-AH	=	Platelet activating factor-acetylhydase
PC	=	Phosphatidylcholine
PDGF	=	Platelet derived growth factor
PGI ₂	=	Prostacyclin
PI-3K	=	phosphoinositol-3 kinase
PL A ₂	=	Phospholipase A ₂

PLD = Phospholipase D
 PKC = Protein kinase C
 PTK = Protein tyrosine kinase
 ROS = Reactive oxygen species
 RTK = Receptor tyrosine kinase
 SDF-1 = Stromal cell-derived factor-1
 siRNA = Small interference RNA
 SMCs = Smooth muscle cells
 SMMP = Smooth muscle myosin phosphatase
 sPL A₂ = Secretory phospholipase A₂
 TRPC5 = transient receptor potential channel 5
 VCAM-1 = Vascular cell adhesion molecule-1
 VSMC = Vascular SMC

REFERENCES

- Diaz, M.N.; Frei, B.; Vita, J.A.; Keane, J.F. Jr. *N. Engl. J. Med.*, **1997**, *337*, 408-416.
- Ross, R. *N. Engl. J. Med.*, **1999**, *340*, 115-126.
- Hansson, G.K. *N. Engl. J. Med.*, **2005**, *352*, 1685-1695.
- Tedgui, A.; Mallat, Z. *Physiol. Rev.*, **2006**, *86*, 515-581.
- Cox, D.A.; Cohen, M.L. *Pharmacol. Rev.*, **1996**, *48*, 3-19.
- Witztum, J.L.; Steinberg, D. *Trends Cardiovasc. Med.*, **2001**, *11*, 93-102.
- Stocker, R.; Keane, J.F. Jr. *Physiol. Rev.*, **2004**, *84*, 1381-1478.
- Steinberg, D. *Nat. Med.*, **2002**, *8*, 1211-1217.
- McIntyre, T.M.; Zimmerman, G.A.; Prescott, S.M. *J. Biol. Chem.*, **1999**, *274*, 25189-25192.
- Berliner, J.A.; Subbanagounder, G.; Leitinger, N.; Watson, A.D.; Vora, D. *Trends Cardiovasc. Med.*, **2001**, *11*, 142-147.
- Tselepis, A.D.; Chapman, M.J. *Atheroscler. Suppl.*, **2002**, *3*, 57-68.
- Schroeksnadel, K.; Frick, B.; Winkler, C.; Fuchs, D. *Curr. Vasc. Pharmacol.*, **2006**, *4*, 205-13.
- Boyle, J.J. *Curr. Vasc. Pharmacol.*, **2005**, *3*, 63-68.
- Pattison, D.I.; Davies, M.J. *Curr. Med. Chem.*, **2006**, *13*, 3271-3290.
- Reiss, A.B.; Vagell, M.E. *Curr. Med. Chem.*, **2006**, *13*, 3227-3238.
- Siasos, G.; Tousoulis, D.; Siasou, Z.; Stefanadis, C.; Papavassiliou, A.G. *Curr. Med. Chem.*, **2007**, *14*, 1567-1572.
- de Leval, X.; Hanson, J.; David, J.L.; Masereel, B.; Pirotte, B.; Dogne, J.M. *Curr. Med. Chem.*, **2004**, *11*, 1243-1252.
- Traupe, T.; Ortmann, J.; Munter, K.; Barton, M. *Curr. Vasc. Pharmacol.*, **2003**, *1*, 111-121.
- Chen, L.; Liang, B.; Froese, D.E.; Liu, S.; Wong, J.T.; Tran, K.; Hatch, G.M.; Mymmin, D.; Kroeger, E.A.; Man, R.Y.; Choy, P.C. *J. Lipid Res.*, **1997**, *38*, 546-553.
- Subbaiah, P.V.; Liu, M. *J. Lipid Res.*, **1996**, *37*, 113-122.
- Dobiasova, M.; Frohlich, J. *Physiol. Res.*, **1998**, *47*, 387-397.
- Wells, I.C.; Peitzmeier, G.; Vincent, J.K. *Exp. Mol. Pathol.*, **1986**, *45*, 303-310.
- McHowat, J.; Corr, P.B. *J. Biol. Chem.*, **1993**, *268*, 15605-15610.
- Kougias, P.; Chai, H.; Lin, P.H.; Lumsden, A.B.; Yao, Q.; Chen, C. *Med. Sci. Monit.*, **2006**, *12*, RA5-RA16.
- Rosengren, B.; Jonsson-Rylander, A.C.; Peilott, H.; Camejo, G.; Hurt-Camejo, E. *Biochim. Biophys. Acta*, **2006**, *1761*, 1301-1308.
- Caslake, M.J.; Packard, C.J.; Suckling, K.E.; Holmes, S.D.; Chamberlain, P.; Macphee, C.H. *Atherosclerosis*, **2000**, *150*, 413-419.
- Zalewski, A.; Macphee, C. *Arterioscler. Thromb. Vasc. Biol.*, **2005**, *25*, 923-931.
- Koenig, W.; Twardella, D.; Brenner, H.; Rothenbacher, D. *Arterioscler. Thromb. Vasc. Biol.*, **2006**, *26*, 1586-1593.
- O'Donoghue, M.; Morrow, D.A.; Sabatine, M.S.; Murphy, S.A.; McCabe, C.H.; Cannon, C.P.; Braunwald, E. *Circulation*, **2006**, *113*, 1745-1752.
- McHowat, J.; Creer, M.H. *Am. J. Physiol.*, **1997**, *272*, H1972-H1980.
- Tokumura, A.; Kanaya, Y.; Kitahara, M.; Miyake, M.; Yoshioka, Y.; Fukuzawa, K. *J. Lipid Res.*, **2002**, *43*, 307-315.
- Mehta, D.; Gupta, S.; Gaur, S.N.; Gangal, S.V.; Agrawal, K.P. *Am. Rev. Respir. Dis.*, **1990**, *142*, 157-161.
- Quinn, M.T.; Parthasarathy, S.; Steinberg, D. *Proc. Natl. Acad. Sci. U. S. A.*, **1988**, *85*, 2805-2809.
- Sonoki, K.; Iwase, M.; Iino, K.; Ichikawa, K.; Ohdo, S.; Higuchi, S.; Yoshinari, M.; Iida, M. *Metabolism*, **2003**, *52*, 308-314.
- Takahara, N.; Kashiwagi, A.; Nishio, Y.; Harada, N.; Kojima, H.; Maegawa, H.; Hidaka, H.; Kikkawa, R. *Diabetologia*, **1997**, *40*, 662-670.
- Rabini, R.A.; Galassi, R.; Fumelli, P.; Dousset, N.; Solera, M.L.; Valdiguie, P.; Curatola, G.; Ferretti, G.; Taus, M.; Mazzanti, L. *Diabetes*, **1994**, *43*, 915-919.
- Zhang, B.; Fan, P.; Shimoji, E.; Itabe, H.; Miura, S.; Uehara, Y.; Matsunaga, A.; Saku, K. *Atherosclerosis*, **2006**, *186*, 291-301.
- Galili, O.; Versari, D.; Sattler, K.J.; Olson, M.L.; Mannheim, D.; McConnell, J.P.; Chade, A.R.; Lerman, L.O.; Lerman, A. *Am. J. Physiol. Heart Circ. Physiol.*, **2007**, *292*, H904-H911.
- Okajima, F.; Sato, K.; Tomura, H.; Kuwabara, A.; Nochi, H.; Tamoto, K.; Kondo, Y.; Tokumitsu, Y.; Ui, M. *Biochem. J.*, **1998**, *336*, 491-500.
- Croset, M.; Brossard, N.; Polette, A.; Lagarde, M. *Biochem. J.*, **2000**, *345*, 61-67.
- Liu, E.; Goldhaber, J.L.; Weiss, J.N. *J. Clin. Invest.*, **1991**, *88*, 1819-1832.
- Saunders, L. *Biochim. Biophys. Acta*, **1966**, *125*, 70-74.
- Mohandas, N.; Wyatt, J.; Mel, S.F.; Rossi, M.E.; Shohet, S.B. *J. Biol. Chem.*, **1982**, *257*, 6537-6543.
- Weltzien, H.U. *Biochim. Biophys. Acta*, **1979**, *559*, 259-287.
- Hara, S.; Shike, T.; Takasu, N.; Mizui, T. *Arterioscler. Thromb. Vasc. Biol.*, **1997**, *17*, 1258-1266.
- Kugiyama, K.; Sakamoto, T.; Misumi, I.; Sugiyama, S.; Ohgushi, M.; Ogawa, H.; Horiuchi, M.; Yasue, H. *Circ. Res.*, **1993**, *73*, 335-343.
- Kohno, M.; Yasunari, K.; Maeda, K.; Kano, H.; Minami, M.; Hanehira, T.; Yoshikawa, J. *Hypertension*, **2000**, *35*, 971-977.
- Vuong, T.D.; de Kimpe, S.; de Roos, R.; Rabelink, T.J.; Koomans, H.A.; Joles, J.A. *Kidney Int.*, **2001**, *60*, 1088-1096.
- McMurray, H.F.; Parthasarathy, S.; Steinberg, D. *J. Clin. Invest.*, **1993**, *92*, 1004-1008.
- Chai, Y.C.; Howe, P.H.; DiCorleto, P.E.; Chisolm, G.M. *J. Biol. Chem.*, **1996**, *271*, 17791-17797.
- Chang, M.Y.; Tsoi, C.; Wight, T.N.; Chait, A. *Arterioscler. Thromb. Vasc. Biol.*, **2003**, *23*, 809-815.
- Liu-Wu, Y.; Hurt-Camejo, E.; Wiklund, O. *Atherosclerosis*, **1998**, *137*, 351-357.
- Oestvang, J.; Anthonsen, M.W.; Johansen, B. *FEBS Lett.*, **2003**, *555*, 257-262.
- Carpenter, K.L.; Dennis, I.F.; Challis, I.R.; Osborn, D.P.; Macphee, C.H.; Leake, D.S.; Arends, M.J.; Mitchinson, M.J. *FEBS Lett.*, **2001**, *503*, 357-363.
- Nishi, E.; Kume, N.; Ueno, Y.; Ochi, H.; Moriwaki, H.; Kita, T. *Circ. Res.*, **1998**, *83*, 508-515.
- Han, K.H.; Hong, K.H.; Ko, J.; Rhee, K.S.; Hong, M.K.; Kim, J.J.; Kim, Y.H.; Park, S.J. *J. Leukoc. Biol.*, **2004**, *76*, 195-202.
- Zurgil, N.; Afrimzon, E.; Shafran, Y.; Shovman, O.; Gilburd, B.; Brikman, H.; Shoefeld, Y.; Deutsch, M. *Atherosclerosis*, **2007**, *190*, 73-83.
- Kume, N.; Ybulsky, M.I.; Gimbrone, M.A. Jr. *J. Clin. Invest.*, **1992**, *90*, 1138-1144.
- Takahara, N.; Kashiwagi, A.; Maegawa, H.; Shiget, Y. *Metabolism*, **1996**, *45*, 559-564.
- Scalia, R.; Murohara, T.; Campbell, B.; Kaji, A.; Lefer, A.M. *Am. J. Physiol.*, **1997**, *272*, H2584-H2590.
- Wong, J.T.; Tran, K.; Pierce, G.N.; Chan, A.C.; O, K.; Choy, P.C. *J. Biol. Chem.*, **1998**, *273*, 6830-6836.
- Rikitake, Y.; Kawashima, S.; Yamashita, T.; Ueyama, T.; Ishido, S.; Hotta, H.; Hirata, K.; Yokoyama, M. *Arterioscler. Thromb. Vasc. Biol.*, **2000**, *20*, 1006-1012.
- Fukao, M.; Hattori, Y.; Kanno, M.; Sakuma, I.; Kitabatake, A. *Biochem. Biophys. Res. Commun.*, **1996**, *227*, 479-483.
- Fukao, M.; Hattori, Y.; Kanno, M.; Sakuma, I.; Kitabatake, A. *Br. J. Pharmacol.*, **1995**, *116*, 1541-1543.
- Matsumoto, T.; Miyamori, K.; Kobayashi, T.; Kamata, K. *Vascul. Pharmacol.*, **2006**, *44*, 450-460.
- Takahashi, M.; Okazaki, H.; Ogata, Y.; Takeuchi, K.; Ikeda, U.; Shimada, K. *Atherosclerosis*, **2002**, *161*, 387-394.
- Rong, J.X.; Berman, J.W.; Taubman, M.B.; Fisher, E.A. *Arterioscler. Thromb. Vasc. Biol.*, **2002**, *22*, 1617-1623.
- Yamakawa, T.; Tanaka, S.; Yamakawa, Y.; Kamei, J.; Numaguchi, K.; Motley, E.D.; Inagami, T.; Eguchi, S. *Arterioscler. Thromb. Vasc. Biol.*, **2002**, *22*, 752-758.
- Kohno, M.; Yokokawa, K.; Yasunari, K.; Minami, M.; Kano, H.; Hanehira, T.; Yoshikawa, J. *Circulation*, **1998**, *98*, 353-359.
- Hsieh, C.C.; Yen, M.H.; Liu, H.W.; Lau, Y.T. *Atherosclerosis*, **2000**, *151*, 481-491.
- Stary, H.C.; Chandler, A.B.; Dinsmore, R.E.; Fuster, V.; Glagov, S.; Insull, W. Jr.; Rosenfeld, M.E.; Schwartz, C.J.; Wagner, W.D.; Wissler, R.W. *Circulation*, **1995**, *92*, 1355-1374.
- Huo, Y.; Ley, K. *Acta Physiol. Scand.*, **2001**, *173*, 35-43.
- McEver, R.P.; Beckstead, J.H.; Moore, K.L.; Marshall-Carlson, L.; Bainton, D.F. *J. Clin. Invest.*, **1989**, *84*, 92-99.
- Lorant, D.E.; Topham, M.K.; Whatley, R.E.; McEver, R.P.; McIntyre, T.M.; Prescott, S.M.; Zimmerman, G.A. *J. Clin. Invest.*, **1993**, *92*, 559-570.

- [80] Ueda, A.; Okuda, K.; Ohno, S.; Shirai, A.; Igarashi, T.; Matsunaga, K.; Fukushima, J.; Kawamoto, S.; Ishigatsubo, Y.; Okubo, T. *J. Immunol.*, **1994**, *153*, 2052-2063.
- [81] Cushing, S.D.; Berliner, J.A.; Valente, A.J.; Territo, M.C.; Navab, M.; Parhami, F.; Gerrity, R.; Schwartz, C.J.; Fogelman, A.M. *Proc. Natl. Acad. Sci. U. S. A.*, **1990**, *87*, 5134-5138.
- [82] Gu, L.; Okada, Y.; Clinton, S.K.; Gerard, C.; Sukhova, G.K.; Libby, P.; Rollins, B.J. *Mol. Cell.*, **1998**, *2*, 275-281.
- [83] Gosling, J.; Slaymaker, S.; Gu, L.; Tseng, S.; Zlot, C.H.; Young, S.G.; Rollins, B.J.; Charo, I.F. *J. Clin. Invest.*, **1999**, *103*, 773-778.
- [84] Boring, L.; Gosling, J.; Cleary, M.; Charo, I.F. *Nature*, **1998**, *394*, 894-897.
- [85] Aiello, R.J.; Bourassa, P.A.; Lindsey, S.; Weng, W.; Natoli, E.; Rollins, B.J.; Milos, P.M. *Arterioscler. Thromb. Vasc. Biol.*, **1999**, *19*, 1518-1525.
- [86] Pan, J.P.; Cheng, T.M.; Chou, S.C.; Lai, S.T. *J. Formos. Med. Assoc.*, **2003**, *102*, 151-157.
- [87] Guest, T.M.; Vlastos, G.; Alameddine, F.M.; Taylor, W.R. *Antioxid. Redox. Signal.*, **2006**, *8*, 1461-1471.
- [88] Libby, P. *Nature*, **2002**, *420*: 868-874.
- [89] Kita, T.; Kume, N.; Yokode, M.; Ishii, K.; Arai, H.; Horiuchi, H.; Moriwaki, H.; Minami, M.; Kataoka, H.; Wakatsuki, Y. *Ann. N. Y. Acad. Sci.*, **2000**, *902*, 95-100.
- [90] Stemme, S.; Holm, J.; Hansson, G.K. *Arterioscler. Thromb.*, **1992**, *12*, 206-211.
- [91] Sakata-Kaneko, S.; Wakatsuki, Y.; Usui, T.; Matsunaga, Y.; Itoh, T.; Nishi, E.; Kume, N.; Kita, T. *FEBS Lett.*, **1998**, *433*, 161-165.
- [92] Abi-Younes, S.; Sauty, A.; Mach, F.; Sukhova, G.K.; Libby, P.; Luster, A.D. *Circ. Res.*, **2000**, *86*, 131-138.
- [93] Bleul, C.C.; Fuhlbrigge, R.C.; Casasnovas, J.M.; Aiuti, A.; Springer, T.A. *J. Exp. Med.*, **1996**, *184*, 1101-1109.
- [94] Nanki, T.; Lipsky, P.E. *J. Immunol.*, **2000**, *164*, 5010-5014.
- [95] Benitez, S.; Camacho, M.; Arcelus, R.; Vila, L.; Bancells, C.; Ordonez-Llanos, J.; Sanchez-Quesada, J.L. *Atherosclerosis*, **2004**, *177*, 299-305.
- [96] Hirata, K.; Miki, N.; Kuroda, Y.; Sakoda, T.; Kawashima, S.; Yokoyama, M. *Circ. Res.*, **1995**, *76*, 958-962.
- [97] Kume, N.; Gimbrone, M.A. Jr. *J. Clin. Invest.*, **1994**, *93*, 907-911.
- [98] Murohara, T.; Scalia, R.; Lefer, A.M. *Circ. Res.*, **1996**, *78*, 780-789.
- [99] Murugesan, G.; Sandhya Rani, M.R.; Gerber, C.E.; Mukhopadhyay, C.; Ransohoff, R.M.; Chisolm, G.M.; Kottke-Marchant, K. *J. Mol. Cell Cardiol.*, **2003**, *35*, 1375-1384.
- [100] Zembowicz, A.; Jones, S.L.; Wu, K.K. *J. Clin. Invest.*, **1995**, *96*, 1688-1692.
- [101] Zembowicz, A.; Tang, J.L.; Wu, K.K. *J. Biol. Chem.*, **1995**, *270*, 17006-17010.
- [102] Cuccurullo, C.; Fazio, M.L.; Mezzetti, A.; Cipollone, F. *Curr. Med. Chem.*, **2007**, *14*, 1595-1605.
- [103] Vane, J.R.; Bakhle, Y.S.; Botting, R.M. *Annu. Rev. Pharmacol. Toxicol.*, **1998**, *38*, 97-120.
- [104] Linton, M.F.; Fazio, S. *Curr. Opin. Pharmacol.*, **2004**, *4*, 116-123.
- [105] Pieper, G.M. *Hypertension*, **1998**, *31*, 1047-1060.
- [106] Braam, B.; Verhaar, M.C. *Curr. Pharm. Des.*, **2007**, *13*, 1727-1740.
- [107] Rabelink, T.J.; Luscher, T.F. *Arterioscler. Thromb. Vasc. Biol.*, **2006**, *26*, 267-271.
- [108] Takeshita, S.; Inoue, N.; Gao, D.; Rikitake, Y.; Kawashima, S.; Tawa, R.; Sakurai, H.; Yokoyama, M. *J. Atheroscler. Thromb.*, **2000**, *7*, 238-246.
- [109] Kugiyama, K.; Sugiyama, S.; Ogata, N.; Oka, H.; Doi, H.; Ota, Y.; Yasue, H. *Atherosclerosis*, **1999**, *143*, 201-204.
- [110] Cieslik, K.; Zembowicz, A.; Tang, J.L.; Wu, K.K. *J. Biol. Chem.*, **1998**, *273*, 14885-14890.
- [111] Cieslik, K.; Abrams, C.S.; Wu, K.K. *J. Biol. Chem.*, **2001**, *276*, 1211-1219.
- [112] Bouloumie, A.; Bauersachs, J.; Linz, W.; Scholkens, B.A.; Wiemer, G.; Fleming, I.; Busse, R. *Hypertension*, **1997**, *30*, 934-941.
- [113] Laursen, J.B.; Somers, M.; Kurz, S.; McCann, L.; Warnholtz, A.; Freeman, B.A.; Tarpey, M.; Fukai, T.; Harrison, D.G. *Circulation*, **2001**, *103*, 1282-1288.
- [114] Hink, U.; Li, H.; Mollnau, H.; Oelze, M.; Matheis, E.; Hartmann, M.; Skatchkov, M.; Thaiss, F.; Stahl, R.A.; Warnholtz, A.; Meinertz, T.; Griendling, K.; Harrison, D.G.; Forstermann, U.; Munzel, T. *Circ. Res.*, **2001**, *88*, E14-E22.
- [115] Kugiyama, K.; Ohgushi, M.; Sugiyama, S.; Murohara, T.; Fukunaga, K.; Miyamoto, E.; Yasue, H. *Circ. Res.*, **1992**, *71*, 1422-1428.
- [116] Ohgushi, M.; Kugiyama, K.; Fukunaga, K.; Murohara, T.; Sugiyama, S.; Miyamoto, E.; Yasue, H. *Arterioscler. Thromb.*, **1993**, *13*, 1525-1532.
- [117] Sugiyama, S.; Kugiyama, K.; Ohgushi, M.; Fujimoto, K.; Yasue, H. *Circ. Res.*, **1994**, *74*, 565-575.
- [118] Fang, X.; Gibson, S.; Flowers, M.; Furui, T.; Bast, R.C. Jr.; Mills, G.B. *J. Biol. Chem.*, **1997**, *272*, 13683-13689.
- [119] Adhikari, N.; Charles, N.; Lehmann, U.; Hall, J.L. *Curr. Atheroscler. Rep.*, **2006**, *8*, 252-260.
- [120] Sugiyama, S.; Kugiyama, K.; Ogata, N.; Doi, H.; Ota, Y.; Ohgushi, M.; Matsumura, T.; Oka, H.; Yasue, H. *Arterioscler. Thromb. Vasc. Biol.*, **1998**, *18*, 568-576.
- [121] Chisolm, G.M. 3rd; Chai, Y. *Free Radic. Biol. Med.*, **2000**, *28*, 1697-1707.
- [122] Aiyar, N.; Disa, J.; Ao, Z.; Ju, H.; Nerurkar, S.; Willette, R.N.; Macphee, C.H.; Johns, D.G.; Douglas, S.A. *Mol. Cell Biochem.*, **2007**, *295*, 113-120.
- [123] Chai, Y.C.; Howe, P.H.; DiCorleto, P.E.; Chisolm, G.M. *J. Biol. Chem.*, **1996**, *271*, 17791-17797.
- [124] Littlewood, T.D.; Bennett, M.R. *Curr. Opin. Lipidol.*, **2003**, *14*, 469-475.
- [125] Lauber, K.; Bohn, E.; Krober, S.M.; Xiao, Y.J.; Blumenthal, S.G.; Lindemann, R.K.; Marini, P.; Wiedig, C.; Zobywalski, A.; Baksh, S.; Xu, Y.; Autenrieth, I.B.; Schulze-Osthoff, K.; Belka, C.; Stuhler, G.; Wesselberg, S. *Cell*, **2003**, *113*, 717-730.
- [126] Matsubara, M.; Hasegawa, K. *Atherosclerosis*, **2005**, *178*, 57-66.
- [127] Goonesinghe, A.; Mundy, E.S.; Smith, M.; Khosravi-Far, R.; Martinou, J.C.; Esposti, M.D. *Biochem. J.*, **2005**, *387*, 109-118.
- [128] Cai, H.; Harrison, D.G. *Circ. Res.*, **2000**, *87*, 840-844.
- [129] Matsumoto, T.; Kakami, M.; Noguchi, E.; Kobayashi, T.; Kamata, K. *Am. J. Physiol. Heart Circ. Physiol.*, **2007**, *293*, H1480-H1490.
- [130] Landmesser, U.; Hornig, B.; Drexler, H. *Circulation*, **2004**, *109*, II27-II33.
- [131] Kobayashi, T.; Taguchi, K.; Yasuhiro, T.; Matsumoto, T.; Kamata, K. *Hypertension*, **2004**, *44*, 956-962.
- [132] Da Ros, R.; Assaloni, R.; Ceriello, A. *Curr. Vasc. Pharmacol.*, **2004**, *2*, 335-341.
- [133] Boak, L.; Chin-Dusting, J.P. *Curr. Vasc. Pharmacol.*, **2004**, *2*, 45-52.
- [134] Ihling, C.; Bohrmann, B.; Schaefer, H.E.; Technau-Ihling, K.; Loeffler, B.M. *Curr. Vasc. Pharmacol.*, **2004**, *2*, 249-258.
- [135] Kobayashi, T.; Matsumoto, T.; Kamata, K. *J. Smooth Muscle Res.*, **2005**, *41*, 283-302.
- [136] Haidara, M.A.; Yassin, H.Z.; Rateb, M.; Ammar, H.; Zorkani, M.A. *Curr. Vasc. Pharmacol.*, **2006**, *4*, 215-227.
- [137] Matsumoto, T.; Miyamori, K.; Kobayashi, T.; Kamata, K. *Free Radic. Biol. Med.*, **2006**, *41*, 1289-1303.
- [138] Matsumoto, T.; Kobayashi, T.; Kamata, K. *Curr. Cardiol. Rev.*, **2006**, *2*, 185-191.
- [139] Matsumoto, T.; Noguchi, E.; Kobayashi, T.; Kamata, K. *Free Radic. Biol. Med.*, **2007**, *42*, 993-1007.
- [140] Lahera, V.; Goicoechea, M.; de Vinuesa, S.G.; Miana, M.; de las Heras, N.; Cachofeiro, V.; Luno, J. *Curr. Med. Chem.*, **2007**, *14*, 243-248.
- [141] Schulz, E.; Anter, E.; Keane, J.F. Jr. *Curr. Med. Chem.*, **2004**, *11*, 1093-1104.
- [142] Matsumoto, T.; Kobayashi, T.; Kamata, K. *Am. J. Physiol. Heart Circ. Physiol.*, **2003**, *285*, H283-H291.
- [143] Yanga, Q.; Yima, A.P.; He, G.W. *Curr. Vasc. Pharmacol.*, **2007**, *5*, 85-92.
- [144] Flavahan, N.A. *Am. J. Physiol.*, **1993**, *264*, H722-H727.
- [145] Kamata, K.; Nakajima, M. *Br. J. Pharmacol.*, **1998**, *123*, 1509-1516.
- [146] Froese, D.E.; McMaster, J.; Man, R.Y.; Choy, P.C.; Kroeger, E.A. *Mol. Cell Biochem.*, **1999**, *197*, 1-6.
- [147] Murohara, T.; Kugiyama, K.; Ohgushi, M.; Sugiyama, S.; Ohta, Y.; Yasue, H. *Am. J. Physiol.*, **1994**, *267*, H2441-H2449.
- [148] Cowan, C.L.; Steffen, R.P. *Arterioscler. Thromb. Vasc. Biol.*, **1995**, *15*, 2290-2297.
- [149] Kikuta, K.; Sawamura, T.; Miwa, S.; Hashimoto, N.; Masaki, T. *Circ. Res.*, **1998**, *83*, 1088-1096.
- [150] Freeman, J.E.; Kuo, W.Y.; Drenger, B.; Barnett, T.N.; Levine, M.A.; Flavahan, N.A. *J. Cardiovasc. Pharmacol.*, **1996**, *28*, 345-352.
- [151] Cox, D.A.; Cohen, M.L. *Am. J. Physiol.*, **1996**, *271*, H1706-H1710.
- [152] Cox, D.A.; Cohen, M.L. *J. Pharmacol. Exp. Ther.*, **1997**, *283*, 305-311.
- [153] Oishi, K.; Raynor, R.L.; Charp, P.A.; Kuo, J.F. *J. Biol. Chem.*, **1988**, *263*, 6865-6871.
- [154] Ohara, Y.; Peterson, T.E.; Zheng, B.; Kuo, J.F.; Harrison, D.G. *Arterioscler. Thromb.*, **1994**, *14*, 1007-1013.
- [155] Inoue, N.; Takeshita, S.; Gao, D.; Ishida, T.; Kawashima, S.; Akita, H.; Tawa, R.; Sakurai, H.; Yokoyama, M. *Atherosclerosis*, **2001**, *155*, 45-52.
- [156] Locher, R.; Weisser, B.; Mengden, T.; Brunner, C.; Vetter, W. *Biochem. Biophys. Res. Commun.*, **1992**, *183*, 156-162.
- [157] Stoll, L.L.; Spector, A.A. *Am. J. Physiol.*, **1993**, *264*, C885-C893.
- [158] Chen, Y.; Morimoto, S.; Kitano, S.; Koh, E.; Fukuo, K.; Jiang, B.; Chen, S.; Yasuda, O.; Hirohata, A.; Ogihara, T. *Atherosclerosis*, **1995**, *112*, 69-76.
- [159] Terasawa, K.; Nakajima, T.; Iida, H.; Iwasawa, K.; Oonuma, H.; Jo, T.; Morita, T.; Nakamura, F.; Fujimori, Y.; Toyo-oka, T.; Nagai, R. *Circulation*, **2002**, *106*, 3111-3119.
- [160] Jensen, P.E.; Ohanian, J.; Stausbol-Gron, B.; Buus, N.H.; Aalkjaer, C. *Br. J. Pharmacol.*, **1996**, *117*, 1238-1244.
- [161] Zhang, R.; Rodrigues, B.; MacLeod, K.M. *J. Pharmacol. Exp. Ther.*, **2006**, *317*, 355-361.
- [162] Suenaga, H.; Kamata, K. *Eur. J. Pharmacol.*, **1998**, *361*, 217-226.
- [163] Hollenberg, M.D. *Trends Pharmacol. Sci.*, **1994**, *15*, 108-114.
- [164] Touyz, R.M.; Schiffrin, E.L. *Pharmacol. Rev.*, **2000**, *52*, 639-672.
- [165] Roberts, R.E. *Br. J. Pharmacol.*, **2001**, *133*, 859-866.
- [166] Nakashima, H.; Suzuki, H.; Ohtsu, H.; Chao, J.Y.; Utsunomiya, H.; Frank, G.D.; Eguchi, S. *Curr. Vasc. Pharmacol.*, **2006**, *4*, 67-78.
- [167] Yamakawa, T.; Eguchi, S.; Yamakawa, Y.; Motley, E.D. *Hypertension*, **1998**, *31*, 248-253.
- [168] Gouni-Berthold, I.; Sachinidis, A. *Curr. Vasc. Pharmacol.*, **2004**, *2*, 363-370.
- [169] Bassa, B.V.; Roh, D.D.; Vaziri, N.D.; Kirschenbaum, M.A.; Kamanna, V.S. *Am. J. Physiol.*, **1999**, *277*, F328-F337.
- [170] Ozaki, H.; Ishii, K.; Arai, H.; Kume, N.; Kita, T. *Atherosclerosis*, **1999**, *143*, 261-216.
- [171] Suenaga, H.; Kamata, K. *Br. J. Pharmacol.*, **2002**, *135*, 789-799.
- [172] Suenaga, H.; Kamata, K. *J. Pharmacol. Sci.*, **2003**, *92*, 348-358.
- [173] Matsumoto, T.; Kobayashi, T.; Kamata, K. *Br. J. Pharmacol.*, **2006**, *149*, 931-941.

- [174] Somlyo, A.P.; Somlyo, A.V. *Nature*, **1994**, *372*, 231-236.
- [175] Bolz, S.S.; Galle, J.; Derwand, R.; de Wit, C.; Pohl, U. *Circulation*, **2000**, *102*, 2402-2410.
- [176] Chrissobolis, S.; Sobey, C.G. *Circ. Res.*, **2001**, *88*, 774-779.
- [177] Essler, M.; Retzer, M.; Bauer, M.; Heemskerck, J.W.; Aepfelbacher, M.; Siess, W. *J. Biol. Chem.*, **1999**, *274*, 30361-30364.
- [178] Galle, J.; Mameghani, A.; Bolz, S.S.; Gambaryan, S.; Gorg, M.; Quaschnig, T.; Raff, U.; Barth, H.; Seibold, S.; Wanner, C.; Pohl, U. *J. Am. Soc. Nephrol.*, **2003**, *14*, 1471-1479.
- [179] Yeon, D.; Kwon, S.; Nam, T.; Ahn, D. *J. Vet. Med. Sci.*, **2001**, *63*, 395-399.
- [180] Aiello, E.A.; Clement-Chomienne, O.; Sontag, D.P.; Walsh, M.P.; Cole, W.C. *Am. J. Physiol.*, **1996**, *271*, H109-H119.
- [181] Leblanc, N.; Wan, X.; Leung, P.M. *Am. J. Physiol.*, **1994**, *266*, C1523-C1537.
- [182] Nelson, M.T.; Patlak, J.B.; Worley, J.F.; Standen, N.B. *Am. J. Physiol.*, **1990**, *259*, C3-C18.
- [183] Rikitake, Y.; Kawashima, S.; Takahashi, T.; Ueyama, T.; Ishido, S.; Inoue, N.; Hirata, K.; Yokoyama, M. *Am. J. Physiol. Heart Circ. Physiol.*, **2001**, *281*, H266-H274.
- [184] Rikitake, Y.; Hirata, K.; Kawashima, S.; Takeuchi, S.; Shimokawa, Y.; Kojima, Y.; Inoue, N.; Yokoyama, M. *Biochem. Biophys. Res. Commun.*, **2001**, *281*, 1291-1297.
- [185] Hou, M.; Xia, M.; Zhu, H.; Wang, Q.; Li, Y.; Xiao, Y.; Zhao, T.; Tang, Z.; Ma, J.; Ling, W. *Cell Biochem. Funct.*, **2007**, *25*, 33-44.
- [186] Zhu, K.; Baudhuin, L.M.; Hong, G.; Williams, F.S.; Cristina, K.L.; Kabarowski, J.H.; Witte, O.N.; Xu, Y. *J. Biol. Chem.*, **2001**, *276*, 41325-41335.
- [187] Kabarowski, J.H.; Zhu, K.; Le, L.Q.; Witte, O.N.; Xu, Y. *Science*, **2001**, *293*, 702-705.
- [188] Xu, Y. *Biochim. Biophys. Acta*, **2002**, *1582*, 81-88.
- [189] Rikitake, Y.; Hirata, K.; Yamashita, T.; Iwai, K.; Kobayashi, S.; Itoh, H.; Ozaki, M.; Ejiri, J.; Shiomi, M.; Inoue, N.; Kawashima, S.; Yokoyama, M. *Arterioscler. Thromb. Vasc. Biol.*, **2002**, *22*, 2049-2053.
- [190] Wang, L.; Radu, C.G.; Yang, L.V.; Bentolila, L.A.; Riedinger, M.; Witte, O.N. *Mol. Biol. Cell*, **2005**, *16*, 2234-2247.
- [191] Yang, L.V.; Radu, C.G.; Wang, L.; Riedinger, M.; Witte, O.N. *Blood*, **2005**, *105*, 1127-1134.
- [192] Radu, C.G.; Yang, L.V.; Riedinger, M.; Au, M.; Witte, O.N. *Proc. Natl. Acad. Sci. U. S. A.*, **2004**, *101*, 245-250.
- [193] Parks, B.W.; Gambill, G.P.; Lusic, A.J.; Kabarowski, J.H. *J. Lipid Res.*, **2005**, *46*, 1405-1415.
- [194] Parks, B.W.; Lusic, A.J.; Kabarowski, J.H. *Arterioscler. Thromb. Vasc. Biol.*, **2006**, *26*, 2703-2709.
- [195] Bolick, D.T.; Whetzel, A.M.; Skafien, M.; Deem, T.L.; Lee, J.; Hedrick, C.C. *Circ. Res.*, **2007**, *100*, 572-580.
- [196] Lum, H.; Qiao, J.; Walter, R.J.; Huang, F.; Subbaiah, P.V.; Kim, K.S.; Holiian, O. *Am. J. Physiol. Heart Circ. Physiol.*, **2003**, *285*, H1786-H1789.
- [197] Qiao, J.; Huang, F.; Naikawadi, R.P.; Kim, K.S.; Said, T.; Lum, H. *Am. J. Physiol. Lung Cell Mol. Physiol.*, **2006**, *291*, L91-L101.
- [198] Huang, F.; Mehta, D.; Predescu, S.; Kim, K.S.; Lum, H. *Endothelium*, **2007**, *14*, 25-34.
- [199] Prenzel, N.; Zwick, E.; Daub, H.; Leserer, M.; Abraham, R.; Wallasch, C.; Ullrich, A. *Nature*, **1999**, *402*, 884-888.
- [200] Ushio-Fukai, M.; Griendling, K.K.; Becker, P.L.; Hilenski, L.; Halleran, S.; Alexander, R.W. *Arterioscler. Thromb. Vasc. Biol.*, **2001**, *21*, 489-495.
- [201] Lusic, A.J. *Nature*, **2000**, *407*, 233-241.
- [202] Fujita, Y.; Yoshizumi, M.; Izawa, Y.; Ali, N.; Ohnishi, H.; Kanematsu, Y.; Ishizawa, K.; Tsuchiya, K.; Tamaki, T. *Endocrinology*, **2006**, *147*, 1377-1385.
- [203] Inoue, M.; Itoh, H.; Ueda, M.; Naruko, T.; Kojima, A.; Komatsu, R.; Doi, K.; Ogawa, Y.; Tamura, N.; Takaya, K.; Igaki, T.; Yamashita, J.; Chun, T.H.; Masatsugu, K.; Becker, A.E.; Nakao, K. *Circulation*, **1998**, *98*, 2108-2116.
- [204] Choy, P.C.; Tran, K.; Hatch, G.M.; Kroeger, E.A. *Prog. Lipid Res.*, **1997**, *36*, 85-101.
- [205] Prokazova, N.V.; Zvezdina, N.D.; Korotaeva, A.A. *Biochemistry (Mosc)*, **1998**, *63*, 31-37.
- [206] Corr, P.B.; Yamada, K.A. *Herz*, **1995**, *20*, 156-168.
- [207] Flemming, P.K.; Dedman, A.M.; Xu, S.Z.; Li, J.; Zeng, F.; Naylor, J.; Benham, C.D.; Bateson, A.N.; Muraki, K.; Beech, D.J. *J. Biol. Chem.*, **2006**, *281*, 4977-4982.

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