



Review Article

## Vascular Endothelium Dysfunction and Hypertension: Insight on Molecular Basics

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### Abstract

Vascular endothelium dysfunction is the hallmark of hypertension. Vascular endothelium regulates the vascular tone, maintains free flow of blood in vessels and plays a critical role in the mechanics of blood flow, regulation of coagulation, vascular smooth muscle cell growth, inflammation and immune functions. Vascular endothelium dysfunction is characterized by the shifts of endothelial actions towards reduced vasodilatation, pro-inflammatory and pro-thrombotic state that contributes to excessive cell proliferation, impaired apoptosis leading to structural remodeling and hypertension. The considerable progress in understanding of molecular mechanisms of hypertension is not well studied. The contribution of vascular endothelium is essential to discern the molecular interventions. Thus in this review article the molecular pathogenesis of vascular endothelial dysfunction is delineated to provide potential therapeutics to prevent hypertension.

**Keywords:** Endothelium, vascular endothelium dysfunction, hypertension, oxidative stress

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### 1. Introduction

The Endothelium is a large paracrine organ in the body. The endothelium has long been viewed as an inert cellophane-like membrane that lines the circulatory system with its primary essential function being the maintenance of vessel wall permeability [1]. The endothelium provides a cellular lining to all blood vessels in the circulatory system, and forms a structural barrier between the vascular space and the tissues. This cellular layer is no longer viewed as an inert structure, but rather

has been recognized to be a dynamic organ, important in several house-keeping functions in health and disease. In adults, the endothelium weighs approximately 1 kg, comprises  $1.6 \times 10^{13}$  cells and has a surface area between  $1-7\text{m}^2$  [2]. The endothelial cell (EC) is between  $25-50\ \mu\text{m}$  in length,  $10-15\ \mu\text{m}$  in width and up to  $5\ \mu\text{m}$  in depth. Each EC comes into contact with numerous smooth muscle cells and vice versa [3]. Endothelial cells secrete the vasodilator (Nitric oxide (NO) and Prostacyclin (PGI<sub>2</sub>)) and vasoconstrictor (Endothelin-1 (ET-1), Thromboxane A<sub>2</sub> and Platelet activating factor

(PAF)) molecules. NO is a potent vasodilator and possesses many antiatherogenic properties [4]. Endothelial cells are able to produce both vasoconstrictive and vasodilating substances. The main endothelium-derived relaxing factors (EDRF) are nitric oxide, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF). One of the most important regulatory and vasoactive substances produced by the endothelial cells is nitric oxide. The discovery in 1980 of the endothelium's ability to elicit vasodilation led to a revolution in vascular biology [5]. NO is produced in the endothelium by the endothelial nitric oxide synthase (eNOS), which, along with inducible NOS and neuronal NOS, belongs to a family of arginine hydroxylases [6]. NO is reported to inhibit inflammation, VSMC proliferation, platelet adhesion, and tissue factor release. NO is the key endothelium-derived relaxing factor that plays a pivotal role in the maintenance of vascular tone and reactivity. In addition to being the main determinant of basal vascular smooth muscle tone, Nitric Oxide (NO) is the primary endogenous vasodilator [7]. In healthy human subjects, inhibition of NO synthase by N monomethyl-L-arginine acutely increased BP, peripheral vascular resistance, and fractional excretion of Na<sup>+</sup>110. NO is tonically active in the medullary circulation, so that reducing NO production or vascular responsiveness, reportedly enhances the pressure natriuresis response, followed by reductions in papillary blood flow, renal interstitial hydrostatic pressure, and Na<sup>+</sup> excretion by almost 30%, without corresponding changes in total or cortical renal blood flow and glomerular filtration rate [8]. This mechanism may contribute to the blunted pressure natriuresis reported in experimental models. Endothelium derived hyperpolarizing factor (EDHF) opens calcium activated K<sup>+</sup> channels in vascular smooth muscle cells to produce vasorelaxation [9]. Angiotensin II by binding it receptor AT<sub>2</sub> produces bradykinin (Tsutsumi *et al.*, 1999) which stimulates eNOS and consequently increase the formation nitric oxide to produce vasorelaxation [10]. The vascular

endothelium releases adenosine to produce vasorelaxation through activation purinergic (P<sub>2</sub>) receptors [11]. However, release of adenine nucleotides from vascular endothelium produce vasoconstriction through activation of P<sub>2</sub> receptors located on vascular smooth muscle cells [12]. Endothelins are synthesized as preproendothelin which is converted to endothelin-1, 2 or 3 by endothelin converting enzyme [13]. Endothelin-1 produces vasoconstriction through activation of L-type Ca<sup>2+</sup> channel by binding to ET-A receptors on vascular smooth muscle cells. On the other hand, Endothelin-1 produces vasorelaxation by binding it ET-B receptors on endothelial cells [14]. The release of endothelins from endothelium is inversely modulated by release of NO [15]. The activation of prostaglandin endoperoxidase synthase initiate the formation of prostanoids such as PGD<sub>2</sub> and PGF<sub>2</sub> in vascular endothelium which are documented to modulate intracellular Ca<sup>2+</sup> concentration and produce vasoconstriction [16]. In conclusion vascular endothelium is noted to produce vasodilators and vasoconstrictor substances which are responsible to regulate the tone of vascular smooth muscle [17].

**Vascular endothelium:** Regulatory contributors

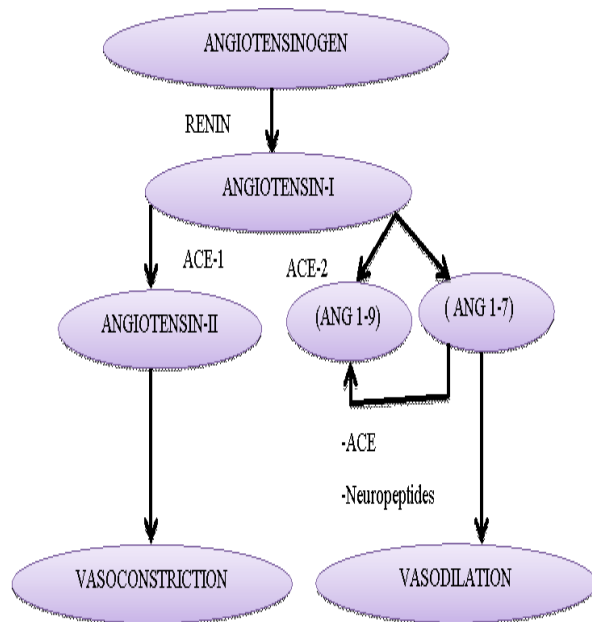
**Neurogenic control:** The vasomotor centre includes the nucleus tractus solitarius in the dorsal medulla (integration of baroreceptor afferents), the rostral part of the ventral medulla (pressor region), the ventrolateral medullary centers (depressor function) and other centers in the pons and mid-brain. Hypothalamus, amygdala and cerebral cortex are also important. The arterial baroreceptors respond to vessel wall distension by increasing the afferent impulse activity. This in turn decreases efferent sympathetic activity and augments vagal tone, resulting in arterial dilation and bradycardia. In hypertension, baroreceptor reflexes are reset, i.e. the range of pressure over which they respond is elevated. In addition, there is a reduction in sensitivity to changes in blood pressure [18,19].

**Renin-angiotensin system:**

Renin is a protease that cleaves angiotensinogen to yield an inactive peptide, angiotensin I. The latter is activated by the angiotensin-converting enzyme (ACE) into the active octapeptide angiotensin [20]. The main source of renin is the juxtaglomerular apparatus of the kidney, which senses changes in renal perfusion pressure and changes in sodium concentration in the distal tubule fluid. In addition to local environmental changes, renin secretion is increased by beta-adrenoceptor stimulation and decreased by alpha-adrenoceptor stimulation. A negative feedback mechanism also exists: high levels of angiotensin II suppress renin secretion. Many tissues other than the kidneys synthesize renin and can produce angiotensin I. These include the liver, brain, adrenal glands, aorta, heart and testicles [21]. Angiotensin I causes local vasoconstriction, modulates mineralocorticoid secretion and contributes to left ventricular hypertrophy. Angiotensin II acts on specific receptors (AT1 and AT2), causing vascular smooth muscle contraction as well as aldosterone, prostacyclin and catecholamine release [22].

RAAS is a major contributor for development of hypertension [75].

**Figure 1:** Flow Chart of Renin angiotensin system



It has been well documented that beneficial effects of ACE-2/ angiotensin (1-7)/Mass receptor axis gets abolished in hypertensives as ACE expression gets upregulated and ACE-2 expression gets downregulated leading to imbalance in two major axes of RAAS and consequent development of hypertension takes place [76]. Angiotensin (1-7) is an active fragment ,formed within ACE-2 mediated enzymatic breakdown of Ang-II , shows vasodilator response by increasing release of vasodilator prostaglandins, nitric oxide and decreasing Ang-II levels [74]. Further, administration of Ang (1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats. It also shows reduction in cardiac fibrotic changes associated with hypertension. Angiotensin (1-7) is converted into Angitensin (1-9) in presence of ACE, neuropeptides and polypeptides. Thus ACE-2/Angiotensin (1-7) is an emerging target for treatment of hypertension and prevention of end stage renal diseases [75,76].

**Atrial natriuretic peptide:**

Atrial natriuretic peptide (ANP) is a 28 amino acid peptide released from atrial granules. Increased sodium intake increases ANP synthesis and release. ANP levels are increased in hypertension [23]. It produces natriuresis, diuresis and a modest fall of blood pressure, while decreasing plasma renin and aldosterone levels. Plasma cardiac natriuretic peptides and peptide fragments from their molecular precursors are markers of heart disease. Clinical studies have defined the current diagnostic utility of these markers, whereas biochemical elucidation of peptide structure and posttranslational processing has revealed new plasma peptide forms of potential clinical use. Natriuretic propeptide structures undergo variable degrees of endo- and exoproteolytic cleavages as well as amino acid modifications, which leave the plasma phase of the peptides highly heterogeneous and dependent on cardiac pathophysiology and capacity. An ongoing characterization of the molecular heterogeneity

may not only help us to appreciate the biosynthetic capacity of the endocrine heart but may also lead to the discovery of new and more disease-specific targets for future molecular diagnosis. Peptides derived from pro-atrial natriuretic peptide and pro-B-type natriuretic peptides are useful plasma markers in heart failure. New data have defined cardiac myocytes as competent endocrine cells in posttranslational processing and cellular secretion [24].

**Eicosanoids:**

Arachidonic acid metabolites influence blood pressure because of their effects on the blood vessel wall and also because of interactions with the autonomic nervous system, renin-angiotensin system (RAS) and other humoral pathways. While prostaglandin A<sub>2</sub>, PGE<sub>2</sub> and thromboxane A<sub>2</sub> increase blood pressure, PGE<sub>2</sub> and prostacyclin cause vasodilation and decrease blood pressure.

**Kallikrein-kinin system:**

Tissue kallikreins are serine proteases that act upon kininogen to form vasoactive peptides termed kinins, the most important being the vasodilator bradykinin. Kinins play a role in the regulation of renal blood flow as well as sodium and water excretion. Kallikrein excretion is decreased in essential and secondary hypertension [25]. ACE inhibitors decrease the breakdown of kinins, leading to kinin accumulation and vasodilatation. Recent studies suggest the protective role of B<sub>2</sub>R in oxidative

stress-mediated disorders of the heart and also potentiating the activity of KKS can protect against the progression of these diseases, by suppressing oxidative stress presumably via production of NO and PGs. Thus, the cardio- and reno-protective effect of ACE inhibitors could largely exerted by the activation of the KKS [26].

**Adrenal steroids:-**

Mineralocorticoid and glucocorticoids increase blood pressure. Their effects are mediated by sodium and water retention (mineralocorticoids) or by increased vascular reactivity (glucocorticoids). In addition, central receptors may be activated [27,28]

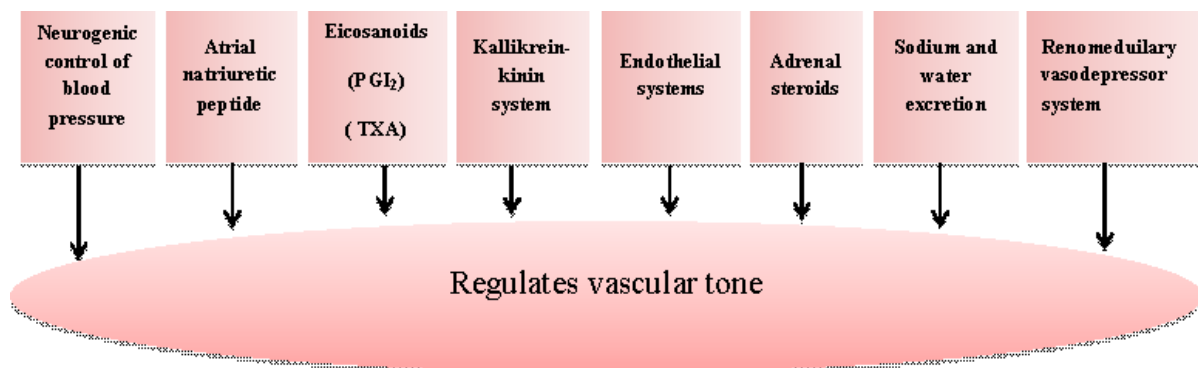
**Renomedullary vasodepressor system:**

The renomedullary interstitial cells, located mainly in the renal papilla, secrete the lipid medullipin I, which is converted by the liver into an active compound, medullipin II. This substance has prolonged blood pressure lowering action, which may be due to the inhibition of the normal sympathetic response to hypotension [29, 30].

**Sodium and water excretion:**

Sodium and water retention are associated with an increase in blood pressure [31,32], possibly because of an increase in intracellular sodium in vascular smooth muscle associated with a secondary increase in intracellular calcium through the sodium calcium exchange mechanism. Sodium retention may also increase neurotransmitter release in the autonomic nervous system. As an increase in blood pressure enhances sodium and water excretion by the

**Figure: 2** Endogenous mechanisms regulating vascular tone and blood pressure



normal kidney, the sodium and water retention in hypertension suggests a shift in the blood pressure-natriuresis relationship. Such a shift may relate to reduce ad renal blood flow, reduced nephron mass, increased angiotensin II levels and/or increased mineralocorticoid levels [33].

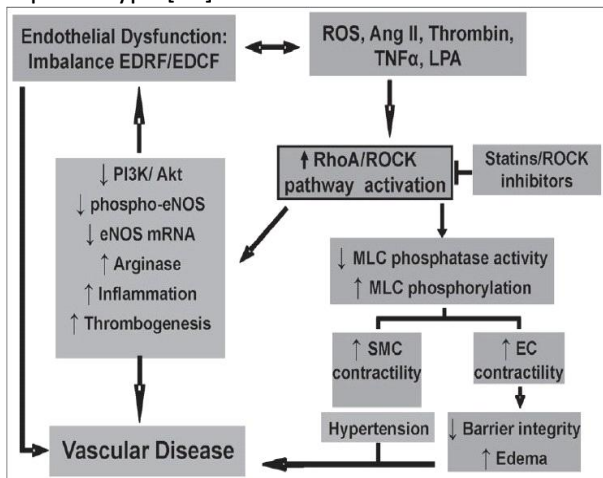
#### **Hypertension and vascular endothelium dysfunction: insightful aspect**

Hypertension is defined as a common disease that is defined simply as persistently elevated arterial blood pressure (BP) above the normal level [34]. It is classified as either primary (essential) or secondary. About 90–95% of cases are termed "primary hypertension", which refers to high blood pressure for which no medical cause can be found [35]. The remaining 5–10% of cases of hypertension are caused by other conditions that affect the kidneys, arteries, heart, or endocrine system [36]. Blood pressure itself is the pressure exerted by the blood on the walls of the blood vessels. Each time the heart beats (about 60-70 times a minute at rest), it pumps blood into the arteries. Blood pressure is at its highest when the heart beats, pumping the blood. That is called systolic blood pressure. When the heart is at rest, between beats, blood pressure falls. This is diastolic pressure. Blood pressure follows a circadian rhythm in a normal individual [37,38]. Hypertension is an important risk factor for cardiovascular accidents, coronary heart disease, cardiac hypertrophy with heart failure (hypertensive heart disease), aortic dissection, and renal failure. Hypertension can also accelerate atherogenesis and can induce changes favorable for aortic dissection and cerebrovascular hemorrhage [6]. The reason for hypertension in 90% of all cases remains unclear termed as essential hypertension. Hypertension is generally a product of genetic predisposition with environmental and lifestyle factors [40]. Genetic predisposition - a family history of hypertension, heart disease, type 2 diabetes, Environmental factors such as age, hormone state and Lifestyle- influential risk factors like smoking, heavy drinking, being

overweight, sodium and calorie-rich diet, lack of physical activity, stress. There is no significant difference in the development of hypertension between men and women although the prevalence of hypertension increases sharply with age, especially for women [41]. Vascular endothelial dysfunction has been implicated in secondary complications due to essential hypertension [42].

Vascular endothelial dysfunction is a functional and reversible alteration of endothelial cells, resulting from impairment in nitric oxide (NO) availability, endothelial cells release NO formed intracellularly by NO Synthase (NOS) from L-arginine in response to several stimuli including increased shear stress during increased blood flow or muscarinic receptor stimulation [43]. Tetrahydrobiopterin deficiency leads to an "uncoupling of NOS" with the resultant production of potent oxidants such as superoxide and hydrogen peroxide. NO opposes the actions of potent endothelium-derived contracting factors such as angiotensin II and endothelin-1 (ET-1). Nitric oxide inhibits platelet and leukocyte activation and maintains the vascular smooth muscle in a non proliferative state [17]. In addition to its production of angiotensin II, prostaglandin endoperoxides, the endothelium is the source of the potent vasoconstrictor peptide ET-1 described as the most potent vasoconstrictor known and acts mainly in a paracrine manner by binding to two G-protein coupled receptors, ETA and ETB, which are located on endothelial cells (ETB), VSMC, and fibroblasts (ETA and ETB). Endothelial ETB receptors can elicit endothelium-dependent relaxation by inducing NO release, whereas ETA and ETB receptors located on smooth muscle cells (SMCs) and fibroblasts trigger vasoconstriction, cell proliferation, inflammation, and fibrosis [44]. Importantly, ET receptor distribution has been shown to be modified in pathological conditions. ET-1 augments the vascular actions of other vasoactive peptides such as angiotensin II, norepinephrine, and serotonin; participates actively in leukocyte and platelet activation; and

facilitates a prothrombotic and proatherogenic phenotype [45].



**Figure 3:** Mechanism involved in hypertension associated vascular endothelium dysfunction.

#### Various target sites underlying vascular endothelium dysfunction in hypertension:

Vascular L- arginine/nitric oxide (NO) synthase system is altered in various pathological disorders such as hypertension, heart failure, hypercholesterolemia and diabetes [46].

#### Asymmetric dimethyl arginine:

It has been shown to accumulate in the plasma in hypercholesterolemia, renal failure and various other peripheral vascular diseases [47]. ADMA is a naturally occurring chemical found in blood plasma. It is a metabolic by-product of continual protein modification processes in the cytoplasm of all cells. It is closely related to L-arginine, a conditionally-essential amino acid. ADMA interferes with L-arginine in the production of nitric oxide, a key chemical involved in normal endothelial function and by extension, cardiovascular health [48]. Asymmetric dimethyl arginine is created in protein methylation, a common mechanism of post-translational protein modification. This reaction is catalyzed by an enzyme set called S-adenosylmethionine protein N-

methyltransferases (protein methylases I and II). The methyl groups transferred to create ADMA are derived from the methyl group donor S-adenosylmethionine, an intermediate in the metabolism of homocysteine. Homocysteine is an important blood chemical, because it is also a marker of cardiovascular disease. After synthesis, ADMA migrates into the extracellular space and then into blood plasma. ADMA may play a role in certain forms of kidney disease with raised levels of ADMA seemingly to be associated with adverse human health consequences for cardiovascular disease, metabolic diseases and also a wide range of diseases of the elderly, the possible lowering of ADMA levels may have important therapeutic effects. ADMA role has been linked with elevated levels of homocysteine [49]. Direct alteration of ADMA levels with supplements of L-arginine has been suggested. The hope is that such intervention might not only improve endothelial function but also reduce clinical symptoms of overt cardiovascular disease [50].

#### Oxidative stress:

Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [51]. Oxidative stress has been implicated causatively in the pathophysiology of many cardiovascular conditions, including hypertension [46]. Superoxide anion is a major determinant of nitric oxide (NO) biosynthesis and bioavailability, and can thus modify endothelial function. It can also act as a vasoconstrictor. In addition, NOS, and in particular the endothelial isoform of NOS (eNOS) is now recognized as an important source of superoxide [52]. The finding that

eNOS can generate superoxide rather than NO in response to atherogenic stimuli has led to the concept of "NOS uncoupling", where the activity of the enzyme for NO production is decreased, in association with an increase in NOS dependent superoxide production [53]. As eNOS may become a peroxynitrite generator, leading to a dramatic increase in oxidative stress, since peroxynitrite formed by the NO superoxide reaction, has additional detrimental effects on vascular function by oxidation of cellular proteins and lipids[54]. Ang-II, acting through the AT1 receptor, stimulates non-phagocytic NADPH oxidase causing the accumulation of superoxide, hydrogen peroxide and peroxynitrite [55].

**Coagulation:**

Ang-II can also upset the balance between the fibrinolytic and coagulation systems via its effect on vascular cells. In particular, Ang II may activate the coagulation cascade by increasing tissue factor (TF) expression in vascular endothelial cells [56,57] or (TF) thrombin activation[58]. In addition, Ang II induces the formation of plasminogen activator inhibitor (PAI)-1 [59,60] an effect that is mediated by specific angiotensin receptors on endothelial cells [61]. AT1, but not AT2 receptor seems to be responsible for the majority of Ang II effects contributing to the thrombosis development [56,61]. More recently, it has been responded that Ang II via AT1 receptor enhances fibrin formation and plasma PAI-1 levels, an effect partially mediated by one Ang IV [62]. Stimulation of PAI-1 synthesis via Ang II production, tissue angiotensin converting enzyme (ACE) also down regulates tissue plasminogen activator (tPA) production via degradation of bradykinin, which is a potent stimulator of tPA production in the endothelium [63]. These actions of tissue

ACE/Ang II on the coagulation/fibrinolytic system can enhance the development of a prothrombotic state [44].

**Adenosine:**

Adenosine, a powerful vasodilating nucleoside, is released during spontaneous or experimental tissue hypoxia circulation and ischemia [64,65]. The vascular endothelium releases adenosine to produce vasorelaxation through activation of purinergic (P<sub>2</sub>) receptors [11]. Adenosine release is tightly related to local oxygen tension [66]. Also adenosine metabolites appear to play a part in local vasoregulation and possibly in the physiological control of blood pressure. Endothelial cell (EC) apoptosis is important in vascular injury, repair, and angiogenesis. Homocysteine and/or adenosine exposure of endothelial cells causes apoptosis. Elevated homocysteine or adenosine occurs in disease states such as homocysteinuria and tissue necrosis, respectively [44].

**Protein tyrosine phosphatase:-**

Protein tyrosine phosphatase (PTPase) is reported to produce apoptotic death of vascular endothelial cells [67]. PTPase which encompasses a family of cytoplasmic and membrane associated enzyme have been implicated in the regulation of cellular proliferation, differentiation and apoptosis [68]. Adenosine activated downstream intracellular mediators PTPase, PTPase mediates the endothelial cells apoptosis by attenuation of growth factors or extracellular matrix. The tyrosine dephosphorylation and the inactivation of MAPK Kinases, solely responsible for endothelial programme cell death is due to PTPase activation. Numerous studies suggest that endothelial cells apoptosis mediated mainly by dephosphorylation and hence decrease P<sub>38</sub> alpha activity [69].

**MAPK (Mitogen Activated Protein Kinases):**

The mitogen activated protein kinases comprise a family of signaling molecules important for coordinating a wide variety of cellular responses. MAPKs play an instrumental role in the transmission of signals from cell surface receptors and of environmental cues to the transcriptional machinery [70]. Members of the MAPK family include ERK1, ERK2, JNK1, and p38. Each of these MAPKs is reversibly activated by the phosphorylation of a conserved threonine and tyrosine motif (TXY). MAPKs are activated in response to extracellular stimuli through dual phosphorylation at conserved threonine and tyrosine residues (serine/threonine specific kinases). In vertebrates, at least three such pathways have been identified; these activate different MAP kinase classes known as extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. MAPKs have key roles in cellular proliferation, differentiation, and apoptosis. Previous studies indicated that ERK1/2 had an opposite role to JNK and p38 in neuronal survival; ERK1/2 promotes neuronal survival, whereas JNK/p38 brings the neuronal cell to apoptosis [71].

**ROCK (Rho Kinase):**

The mandatory role of Rho kinases in numerous cellular function such as vascular smooth muscle contractions actin cytoskeleton cell adhesion cannot be ignored [72]. The upregulation of the various molecule that play pivotal role in inflammation i.e. oxidative stress, thrombus formation and fibrosis by Rho kinase well defines its significance [73]. The phosphorylation of the myosin binding subunit of MLC phosphatase, causes haltation of phosphates activity, which consequently leads to vascular smooth muscle contraction [74,75]. The RhoA/ROCK

pathway activation increases calcium sensitivity in the contraction of the VSMC and modulates the phosphorylation of the myosin light chain (MLC) by inhibiting myosin phosphatase [74,75]. Furthermore, ROCK activation also produces a chain of intracellular events such as endothelial nitric oxidase synthase (eNOS) deregulation (by reducing its gene expression), NADPH oxidase activation with higher oxidative stress that increases gene expression of profibrotic, procoagulant, and proinflammatory genes, and also nuclear factor-kB (NF-kB) activation promoting increased PAI-1 expression and PI3K-Akt inhibition [76]. Currently, there are specific ROCK inhibitors available, such as fasudil and its active metabolite hydroxifasudil [77,78]. Rho kinase have the properties that activate sympathetic nervous system activity, increase the concentration of ACE and Ang II, increase NADPH oxides, increase oxidative stress lead to produce proinflammatory, profibrotic, vasoconstriction as well as inhibit PI3K/AKT and eNOS pathways that produce HT [76]. All these effects are opposite to that produced by Ang (1-7)/Mas-axis activation. Numerous studies have shown that inhibition of Rho kinase, by specific inhibitor (Y-27632) relaxed the rat aortic rings which have been contracted by phenylephrine [79]. The role of Rho kinases in generating ROS attenuates synthesis of NO, promotes permeability of vascular endothelium and causes the contraction and proliferation of vascular smooth muscle cells. The protective role of statins lies in its capacity to inhibit cholesterol production which plays a culprit role in the progression of VED [77]. Thus Rho kinase may play an intricate role in pathogenesis of vascular endothelium [44].



**JAK /STAT (Janus-kinase/signal transducers and activators of transcription factors):**

The Janus-kinase belongs to the family of cytosolic tyrosine kinase and contains four subtypes such as JAK1, JAK2, JAK3 and TYK2 [78]. JAK has been shown to be activated by angiotensin II, serotonin, reactive oxygen species, endothelin-1 and lipopolysaccharide. Activation of JAK pathway has been demonstrated to inhibit PI3-kinase/Akt pathway. The Signaling cascades involved in the transmission of inflammatory processes leading directly from the activated cytokine receptor to gene transcription include the Janus-kinases (JAKs) and signal transducers and activators of transcription (STAT) factors [79, 80]. Recent evidence suggests that the JAK/STAT cascade also conveys angiotensin II (Ang II) signals from the plasma membrane to the nucleus via the stimulation of the Ang II type 1 (AT1) receptor [81]. In addition, the JAK/STAT signaling cascade was shown to be an important link between the activation of the AT1 receptor and nuclear transcriptional changes leading to cell growth [82]. In addition, promoter studies of STAT-regulated genes revealed that STAT-binding sites are in close proximity to binding sites for other transcription factors known to be involved in IL-6 gene transcription, such as nuclear factor-IL-6 [83] and NF-Kb [67]. O<sup>2</sup> anions via the NAD (P) H oxidase [84] are involved in the activation of the JAK/STAT cascade [85].

**PARP (Poly (adenosine diphosphate [ADP]-ribose) polymerase):**

Poly (adenosine diphosphate [ADP]-ribose) polymerase-1 (PARP-1), a monomeric nuclear enzyme present in eukaryotes, is the major isoform of an expanding family of poly (ADP-ribosyl) acting enzymes [86]. PARP-1, main isoform of this family, primarily

functions as a DNA damage sensor in the nucleus. PARP-1 has been implicated in a variety of pathophysiological processes. PARP is an energy-consuming enzyme, which transfers ADP ribose units to nuclear proteins. As a result of this process, the intracellular NAD<sup>+</sup> and adenosine 5'-triphosphate (ATP) levels remarkably decrease, resulting in cell dysfunction and cell death via the necrotic route. PARP becomes activated in response to DNA single-strand breaks, which can develop as a response to free radical and oxidant cell injury [17]. Oxidative and nitrosative stress triggers the activation of the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which contributes to the pathogenesis of various cardiovascular diseases including myocardial infarction and ischemia reperfusion and heart failure, as well as diabetic endothelial dysfunction [87].

**Epoxide hydrolase:**

Epoxyeicosatrienoic acids (EETs) are derived from arachidonic acid by cytochrome P450 epoxygenase, which is a lipid signaling molecule that elicits vasodilatation and modulates various intracellular signaling cascades [88, 89]. The expression of cytochrome P450, which generates EETs in the endothelial cells is not constitutive but is expressed in response to physical and pharmacological stimuli and hence attribute to endothelial derived hyperpolarizing factor (EDHF) [90]. The epoxide hydrolase metabolizes EETs into dihydroxyeicosatrienoic acid (DHETs) and thus reduce the availability of EETs. EETs involve in various physiological functions such as angiogenesis, release of neurohormones, cell proliferation and G protein signaling. Further, EETs significantly inhibit the TNF- $\alpha$  induced caspase-3 activity

and down regulate the proapoptotic Bcl-2 expression and thus prevent apoptotic cell death [91]. Moreover, EET open calcium activated potassium ( $K^+$ ) channels to relax vascular smooth muscle [92]. In addition, EETs up regulate eNOS via PI3K/Akt pathway [93] and hence increase the bioavailability of NO to improve endothelium-dependent vasodilatation. Furthermore, EETs inhibit vascular smooth muscle migration, decrease inflammation, inhibit platelet aggregation and decrease expression of adhesion molecules [94]. Inhibition of epoxide hydrolase is a potential approach to enhance the biological activity of EETs [95]. 1-cyclohexyl-3-dodecylurea (CDU) [94], N, N\_-dicyclohexylurea (DCU) and N-adamantanyl-N\_-dodecanoic acid urea (AUDA) [95] are reported to inhibit epoxide hydrolase. Epoxide hydrolase inhibitors increase the activity of PPAR  $\alpha$  and thus play an important role in lipid metabolism. Epoxide hydrolase inhibitors are found to be useful in the treatment of atherosclerosis [95], hypertension [96], cardiac hypertrophy [97], ischemic injury [98], and inflammatory disease [95] and stroke [98].

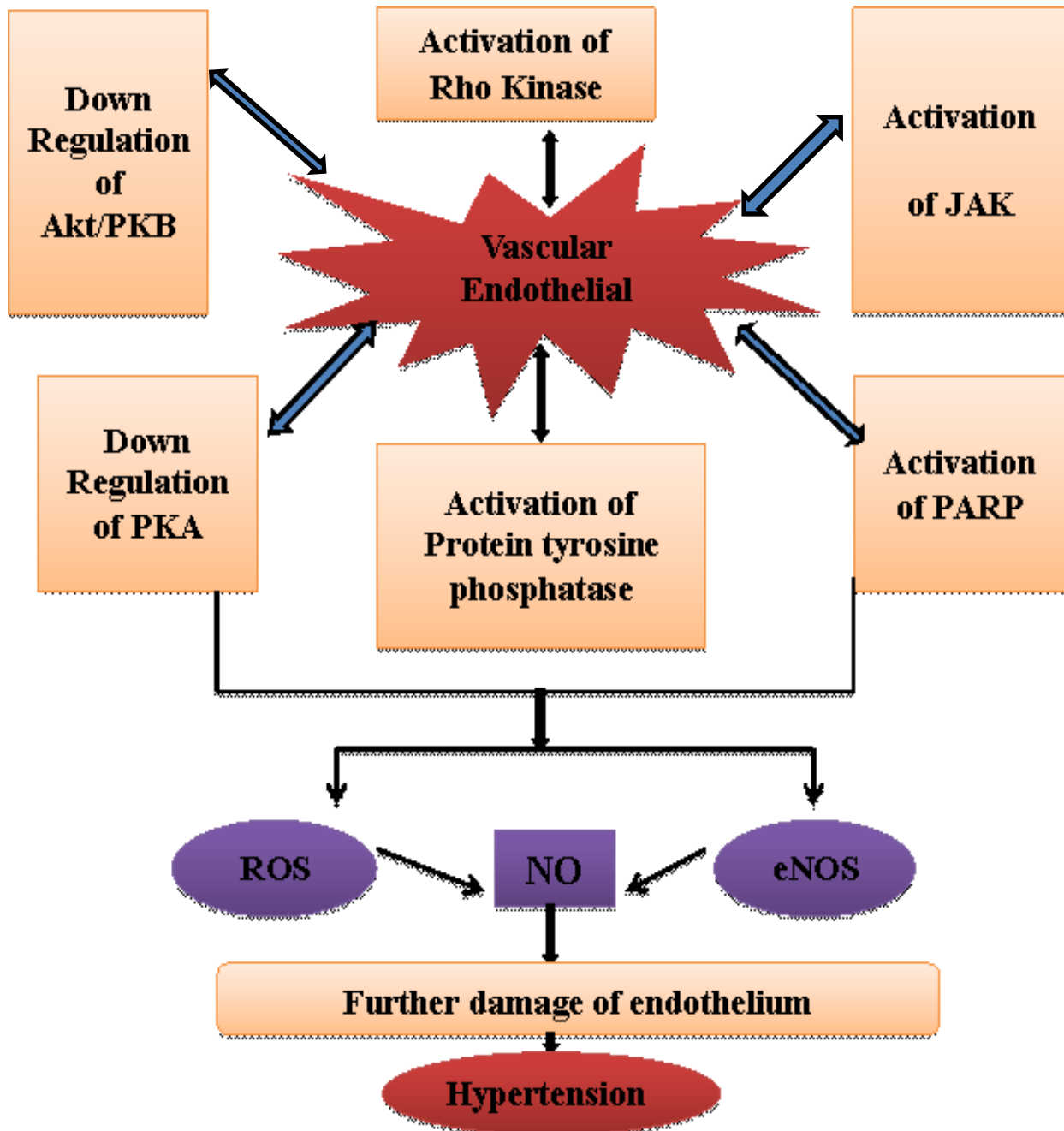
#### **(PI3K) Phosphatidylinositol 3-kinases:**

Phosphatidylinositol 3-kinases (PI3K) are a family of ubiquitously expressed enzymes, which possess both lipid and protein kinase activities [99]. Class I PI-3Ks are enzymes that selectively phosphorylate the 3'-OH position of the inositol in phosphatidylinositol 4, 5-bisphosphate (PIP<sub>2</sub>). Class I PI3Ks have been subdivided further according to their structure and mode of activation by cell surface receptors. The class I a subgroup consist of p110 $\alpha$ , p110 $\beta$  and p110 $\delta$  catalytic subunits associated with a p85 adapter subunit to form a heterodimeric complex [100]. All

three isoforms of this class are activated by the binding of specific phospho-tyrosyl motifs to the two SH<sub>2</sub> domains of the regulatory subunits. Class IA PI3Ks are activated downstream of tyrosine kinase receptor stimulation, such as EGFR (epidermal growth factor receptor) [101]. Class Ib PI3K is composed of the p110 $\gamma$  catalytic subunit associated with a p101 regulatory protein. PI-3K $\gamma$  is stimulated by G $\beta\gamma$  heterodimers released after G protein-coupled receptor (GPCR) activation. Class II PI3Ks contain a C2 domain, which gives their name (PI-3K-C2) to the members of this class of PI3K, and a PX domain (Phox homology domain). This domain is able to bind the two lipid products of PI3K-C2 PtdIns (3) P and PtdIns (3, 4) P<sub>2</sub> to localize these PI3K to the plasma membrane [102]. Class III PI-3Ks contain a single member that is the homologue of *Saccharomyces cerevisiae* Vps34 that generates only PtdIns (3) P [103]. The convenient classification of class I PI-3K isoforms according to their mechanism of activation has been challenged by evidence that PI3K $\beta$  can also be activated by GPCRs [104]. PI-3Ks have been implicated in the modulation of vascular smooth muscle contractility [105]. PI3K $\gamma$  was found to play a role in Ang-II evoked smooth muscle contraction in two crucial signaling pathways that are in response to angiotensin –II; PI3K- $\gamma$  was required for the activation of Rac and the subsequent triggering of ROS production. Conversely, PI3K- $\gamma$  was necessary to activate protein kinase B/Akt, which, in turn, enhanced L-type Ca<sup>2+</sup> channel-mediated extracellular Ca<sup>2+</sup> entry [106]. Although, classes IA and B PI3K isoforms are present in rat portal vein myocytes, injection of antibodies that recognize different PI3K isoforms into these cells indicates that the

angiotensin II-dependent activation of L-type  $Ca^{2+}$  current is inhibited by blocking PI3K- $\gamma$

but not PI3K- $\alpha$  ; this suggests a crucial role for PI3K $\gamma$  in angiotensin II signal transduction



**Figure 4:** Various signaling pathways involved in vascular endothelial dysfunction

[107]. Recent evidence suggests that the vasculotoxic effects of angiotensin II can be

mediated via Phosphatidylinositol 3-kinases signaling pathways [108].

**PPAR-  $\gamma$  (Peroxisome Proliferator Activated Receptor- $\gamma$ ):**

The PPARs belong to a subfamily of the nuclear receptor superfamily and are ligand-activated transcription factors which heterodimerize with the retinoic X receptor and PPAR response elements localized in the promoter region of target genes [109]. To date, three PPAR isoforms have been identified, PPAR- $\alpha$ , PPAR- $\beta/\delta$ , and PPAR- $\gamma$ , with each having similar protein structure despite differences in coding genes [110]. PPAR- $\gamma$  is selectively expressed in various cells such as adipocytes, vascular smooth muscle cells (VSMCs), macrophages as well as in medullary collecting ducts, glomeruli, pelvic urothelium [111] and vascular endothelial cells, that play a role in cell differentiation and lipid and carbohydrate metabolism [112]. PPAR- $\gamma$  modulates Ang II or thromboxane A2 (TxA2) response in the vasculature via transcriptional regulation of their gene or receptor expression. Increased Ang II or TxA2 vasoconstriction and deteriorating renal function observed in glycerol-induced acute renal failure (ARF) may be attributed to a down-regulated PPAR- $\gamma$  expression/activity probably via an increased free radical generation [113]. PPAR- $\gamma$  is expressed in both vascular ECs and VSMCs, making it possible that this receptor plays roles in regulating vascular tone and blood pressure. Indeed, PPAR- $\gamma$  agonists, including thiazolidinediones (TZDs), lower blood pressure in diabetic patients and animal models, at least partially independent of their insulin-sensitizing effects [114], although this blood pressure-lowering effect is much more moderate in human patients than in animal models [115]. Glitazones are high-affinity agonists of peroxisome proliferator-activated receptors (PPARs) presently used in the treatment of type 2 diabetes as efficient insulin sensitizers. These agents lower blood

pressure (BP) in diabetic and hypertensive [116,117], humans and in animal models of renovascular or salt-sensitive hypertension [118, 119] and insulin resistance [120,121]. Although the glitazone-induced improvement in insulin sensitivity is often associated with a decrease in BP [116,117], the mechanisms involved are still unclear. Several studies [122], demonstrate ANG II-antagonizing properties of glitazones are responsible for their antihypertensive effect. However, further studies in this area are warranted [123].

#### **Leptin:**

The leptin is a polypeptide hormone synthesized and secreted into the circulation primarily by white adipocytes. Leptin was identified by positional cloning of the *ob* gene, which determines obesity in *ob/ob* mice. The first described major action of leptin was on the hypothalamus to control body weight and fat deposition through its effects on hypothalamic receptors which leads to appetite inhibition, as well as stimulation of the metabolic rate and thermogenesis. Clinical studies indicate that leptin level is higher in patients with essential hypertension independently of adiposity scores [124]. Elevated leptin is detrimental, especially if it acts for an extended time period. Published data suggest that the enhanced function of the RAAS may be associated with leptin secretion [125]. Indeed, chronic leptin administration or transgenic overexpression increases blood pressure in experimental animals [126] through several mechanisms including: (1) stimulation of sympathetic nervous system [127], (2) impairment of pressure natriuresis [128] and/or induction of sodium retention [129] (3) increased expression of vasoconstrictor ET-1 [130], (4) induction of oxidative stress and NO

deficiency, which results in vasoconstriction and enhanced renal Na<sup>+</sup> reabsorption [131,132]. Mas-knockout (Mas-KO) mice presented dyslipidemia, increased levels of insulin and leptin [133,134] and leptin is very major molecular target which is responsible for maintenance of vascular tone as well as Na<sup>+</sup>/water retention. Some studies indicate that arterial hypertension develops in obese animals only if they are hyperleptinemic [127,135].

**TNF- $\alpha$ :**

TNF- $\alpha$  is an inflammatory cytokine that is synthesized primarily by monocytes and macrophages [136]. The effects of TNF- $\alpha$  are mediated by specific cell surface receptors; an epithelial cell-type receptor (TNF-R1) and a myeloid cell-type receptor (TNF-R2) that is mainly expressed in immune and endothelial cells [137,136]. Aldosterone activates mineralocorticoid receptors (MR) in the periphery and in the central nervous system [138]. Under normal conditions Aldosterone acts primarily to regulate sodium homeostasis by increasing absorption of sodium by the kidneys [139] and stimulating salt appetite [140]. In pathophysiological states characterized by an excess of Aldosterone (e.g., heart failure) [141], stimulation of peripheral MR may induce cardiac remodeling [142], vascular fibrosis [143], and renal injury. Central MR blockade caused an unexpected reduction in the circulating level of the proinflammatory cytokine TNF- $\alpha$  in heart failure rats. It has been shown previously that MR stimulation by systemically administered DOCA also elicits an increase in TNF- $\alpha$  in hypothalamus and pituitary. These increases occurred in a low-renin state and were blocked by the MR antagonist Spironolactone. The effect of DOCA to increase circulating TNF- $\alpha$  in normal rat appears to be mediated entirely by its

effects on MR at the central nervous system level [144].

**Conclusion:**

It has been clear that the process of pathological hypertension is a highly complex event involving various stimuli, membrane receptors, intracellular signaling cascades, transcription factors, genes and effectors. Despite the fact that there have been major advances in the identification of molecular regulators involved in this disease process, but the overall complexity of vascular endothelial dysfunction and hypertension suggests that additional regulatory mechanisms and targets remain to be identified. Thus, further studies are warranted to translate this scientific knowledge into potential pharmacological therapies for the treatment of hypertension and VED. The translation of this knowledge into clinical use will be both exciting and challenging. Hence this review article explains how these targets can be used to cure vascular endothelial dysfunction and hypertension.

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