Roles of MEK/ERK Pathway in Vascular and Renal Tubular Actions of Angiotensin II

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Abstract: Chronic kidney disease (CKD) is now widely recognized as a significant risk factor for cardiovascular disease (CVD). Chronic angiotensin II (Ang II) stimulation facilitates tissue hyperplasia, hypertrophy, and inflammation, and the current medical strategy for CKD is primarily based on the suppression of rein-angiotensin system. Since Ang II induces hypertension through both vasoconstriction and sodium retention, the understanding of vascular and renal actions of Ang II is essential for the better management of CKD and CVD. Ang II is coupled to a variety of intracellular signaling pathways depending on cell types, and Ang II type 1 receptor (AT_1) is thought to be responsible for most, if not all, of the cardiovascular effects of Ang II. Recent studies have suggested that the MEK/ERK pathway plays an important role in Ang II-mediated vascular smooth muscle contraction, where cytosolic phospholipase A_2 ($cPLA_2$)/P450 pathway has a positive feedback effect. Interestingly, the MEK/ERK pathway has been also shown to mediate the stimulatory effect of Ang II on renal proximal transport. However, the $cPLA_2$/P450 pathway has a negative feedback effect on the Ang II-mediated ERK activation in renal proximal tubules. Thus, arachidonic acid metabolites seem to play quite contrasting roles in the Ang II-mediated ERK activation in vascular and renal tissues. This article will be focused on the roles of MEK/ERK pathway in vascular and renal tubular actions of Ang II.

Keywords: Angiotensin II, MEK/ERK pathway, $cPLA_2$, arachidonic acid, P450.

INTRODUCTION

The renin-angiotensin system (RAS) is fundamental for the regulation of cardiovascular system. In particular, angiotensin II (Ang II) is a key hormone in regulation of blood pressure. Although Ang II affects the function of virtually all organs [1], the kidney plays an essential role in the Ang II-induced hypertension [2, 3]. In addition to the vascular effects, Ang II has direct effects on renal tubular functions. Ang II acts on sodium and bicarbonate absorption from proximal tubules, and this process is thought to have a significant impact on body fluid and sodium homeostasis [4, 5]. While acute stimulation with Ang II affects vasoconstriction and solute homeostasis, chronic stimulation facilitates hyperplasia and hypertrophy of vascular smooth muscle cells (VSMCs) [6, 7].

Recently, the involvement of extracellular signal regulated kinase (ERK) pathway in Ang II-mediated vasoconstriction has been recognized [8-11]. The recent study has shown that the ERK pathway is also involved in the Ang II-mediated stimulation of sodium absorption from renal proximal tubules [12]. However, Ang II seems to utilize quite different signaling mechanisms to activate ERK in VSMCs and renal tubules. Since ERK is one of the key factors connecting the acute and chronic effects of Ang II, this review will focus on the roles of ERK pathway in vascular and renal Ang II actions.

CHRONIC Ang II EFFECTS

Ang II is known to activate mitogen-activated protein kinases (MAPKs), such as ERK1/2, JNK and p38MAPK. These pathways may be involved in chronic Ang II actions such as cell differentiation, proliferation, hypertrophy, or migration [1, 13-15]. In addition, Ang II is a potent activator of oxidative stress and oxidant signaling, and reactive oxygen species (ROS) are at least partially responsible for the pleiotrophic effects of Ang II [16-18]. In VSMCs, Ang II activates membrane NAD(P)H oxidase to produce ROS, which facilitate vascular inflammation [18, 19]. In endothelial cells, the Ang II-induced ROS production has been implicated in endothelial dysfunction [16]. The intracellular ROS have been shown to activate transcriptional factor NF-κB, which may result in increased expression of VCAM-1, an important factor in endothelial adhesion [20]. Several modes of cross-talk may exist between MAPKs-dependent and ROS-dependent signaling pathways [1].

Chronic activation of RAS *in vivo* is thought to promote proinflammatory and atherogenic responses through multiple mechanisms, which would eventually result in tissue damages including myocardial infarction, heart failure, stroke, and kidney disease [1, 21]. For example, RAS is considered

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to play pathogenic roles through multiple mechanisms in chronic kidney disease (CKD), a condition associated with increased risk for cardiovascular disease (CVD) [22]. A recent clinical study indeed showed that patients with CKD have a high prevalence of insulin resistance, metabolic syndrome, and chronic inflammation, and that the treatment with an angiotensin receptor blocker (ARB) olmesartan significantly improves insulin resistance as well as inflammation markers in these patients [23].

EFFECTS OF RAS INHIBITION IN CKD

The inhibition of RAS is one of the main therapeutic options in CKD treatment. Previous clinical trials have established the beneficial effects of both angiotensin-converting enzyme (ACE) inhibitors and ARBs in preventing or delaying end-organ damage. Thus, ACE inhibition consistently improved CVD morbidity and mortality rates in patients with heart failure and left ventricular dysfunction [24]. In addition, ACE-inhibitor treatment with captopril significantly reduced loss of renal function in patients with type 1 diabetes [25]. ARB treatment with losartan was also associated with significant reduction in the progression of nephropathy in patients with type 2 diabetes [26]. In view of these beneficial results, a notion becomes wildly accepted that ACE inhibitors and ARBs have tissue protective effects beyond their antihypertensive effects. However, a recent meta-analysis did not support this notion; the beneficial effects of these drugs were attributed only to the reduction of blood pressure [27]. Although this conclusion has drawn much attention and/or criticism, the full understanding of molecular mechanism underlying multiple Ang II actions is obviously indispensable for the establishment of more effective therapeutic strategies for CKD.

Ang II RECEPTORS AND G-PROTEIN COUPLED PATHWAYS

Angiotensin II (Ang II) receptors can be pharmacologically divided into two major subtypes, type 1 (AT_1) and type 2 (AT_2) receptors. AT_1 belongs to the seven-membrane superfamily of G-protein coupled receptors. In rodents, AT_1 receptors are further divided into AT_{1A} and AT_{1B} receptors [28, 29]. While AT_{1A} and AT_{1B} cannot be pharmacologically discriminated [30, 31], AT_{1A} may be more important than AT_{1B} in blood pressure control [32]. While most of the physiological effects of Ang II are mediated by AT_1 receptors [28, 29], AT_2 may also be involved in the regulation of cardiovascular system [33]. In some cases, AT_2 may antagonize the AT_1-mediated signaling *via* serine/threonine phophatases [34]. However, the exact role of AT_2 in Ang II-mediated biological actions has remained still unclear.

AT_1-mediated acute vasoconstriction is mediated by G-protein coupled signaling pathways. Upon stimulation, AT_1 couples to G-protein complexes, which activate downstream effectors such as phospholipase C (PLC) and phospholipase A_2 (PLA_2) [1, 35, 36]. Activation of PLC produces inositol-1,4,5-triphophate (IP_3) and diacylglycerol (DAG). IP_3 increases cell Ca^{2+} concentrations through binding to and opening of its receptor on sarcoplasmic reticulum. Ca^{2+} binds to calmodulin, and triggers actin-myosin interaction, causing smooth muscle cell contraction [1, 37]. In addition to this classical pathway, the ERK pathway may also participate in the Ang II-induced cell Ca^{2+} increase in VSMCs from human resistance arteries [8]. On the other hand, DAG activates PKC, which not only activates the Na^+/H^+ exchanger [38], but also transmits the signal to the Raf/mitogen-activated protein kinase (MEK)/ERK pathway.

ERK PATHWAY IN Ang II ACTIONS ON VASCULAR TONE

A MEK inhibitor PD98059 was shown to significantly attenuate the Ang II-induced increase in blood pressure, indicating that the ERK pathway is involved in the Ang II-mediated acute vasoconstriction [8-11]. The molecular mechanism underlying the ERK-mediated vascular contraction is not completely understood, but phosphorylation of either myosin light chain regulatory subunit or caldesmon may be involved [39, 40]. These targets are considered to increase contraction through modulation of the actin-myosin interaction [41].

There are several proposed models, which link G-protein-coupled receptors to the ERK cascade [1, 15, 42]. They include Ras-dependent ERK activation *via* transactivation of receptor tyrosine kinases such as the epidermal growth factor receptor (EGFR) and platelet derived growth factor receptor (PDGFR), or Ras-independent ERK activation *via* PKC. While EGFR transactivation is thought to be mediated either by release of heparin-binding EGF (HB-EGF) or by cytosolic tyrosine kinase Src, the PKC-dependent ERK activation converges the EGF-dependent signaling at the level of Raf. For example, Eguchi *et al.* showed that the rapid Ang II-induced ERK activation in VSMCs is dependent on cell Ca^{2+} increase as well as Ras and tyrosine kinase activities [43].

In endogenous conditions, however, the transactivation pathway may be less dominant as postulated, whereas pathways involving PKC isoforms may play a more important role as assumed so far [15]. Interestingly, a recent study by Escano *et al.* revealed that Ang II activates ERK through distinct mechanisms in different types of VSMCs [11]. They showed that Ang II activates ERK through the transactivation mechanism in VSMCs from thoracic aorta. In VSMCs from renal microvascular, however, Ang II seems to activate ERK through a unique mechanism, which is not dependent on EGFR or PDGFR, but still dependent on Src.

REGULATION OF RENAL PROXIMAL TRANSPORT BY Ang II

Ang II is one of the most effective activators of renal proximal transport. There are two distinct features in the Ang II actions on renal proximal tubules. Firstly, proximal tubular fluid contains markedly high concentrations of Ang II, which cannot be explained by simple spill over of systemic circulation [44]. In fact, proximal tubular cells have all the components to locally generate Ang II, which, after being secreted into proximal tubular lumen, may exert paracrine/autocrine effects [45, 46]. Secondly, Ang II regulates renal proximal transport in a biphasic manner; stimulation by low (picomolar to nanomolar) concentrations of Ang II and inhibition by high (nanomolar to micromolar) concentrations of Ang II [4, 5].

Traditionally, the stimulatory effect of Ang II has been attributed to the activation of PKC and/or the decrease in the intracellular cAMP level, while the inhibitory effect of Ang II has been attributed to the activation of phospholipase A_2 (PLA_2) and the subsequent release of arachidonic acid [47-49]. Although conflicting data have been reported as to the identity of receptor subtype(s) mediating this unique mode of regulation [50, 51], the data obtained from Ang II type 1 receptor (AT_1)-deficient mice have clearly shown that AT_1 mediates both stimulatory and inhibitory effects of Ang II [52, 53]. This conclusion is consistent with a generally accepted view that the expression of AT_2 is very high in fetal kidney, but rapidly declines soon after birth, and is very low or undetectable in adult kidney [54, 55].

A majority of bicarbonate absorption (or proton secretion) in renal proximal tubules is coupled to sodium absorption. This process is accomplished by the coordinated operation of the apical Na^+/H^+ exchanger NHE3 and the basolateral electrogenic Na^+-HCO_3^- cotransporter NBC1 [56, 57]. Previous studies showed that Ang II stimulates the activities of both NHE3 and NBC1 [52, 58, 59].

ERK PATHWAY IN Ang II ACTIONS ON RENAL PROXIMAL TRANSPORT

In opossum kidney (OK) cells, acidosis was shown to activate the NHE3 activity through Src and MEK/ERK pathways [60]. In OK cells, Ang II was shown to activate the NBC1 activity through the nonreceptor tyrosine kinase proline-rich tyrosine kinase 2 (Pyk2)/Src family kinase coupling, which might be indispensable for the downstream activation of ERK [61]. By contrast, Douglas and colleagues, based on the data obtained from cultured rabbit proximal tubular cells, presented the evidence that the extracellular signal-regulated kinase (ERK) pathway mediates the inhibitory effect of Ang II [50, 62]. In view of these conflicting results, the role of ERK pathway in Ang II-effects on renal transport had not been definitely established.

To clarify this issue, our group examined the role of ERK pathway in Ang II-mediated biphasic regulation of NBC1 activity in isolated mouse proximal tubules. The ARB olmesartan abolished both the stimulation by 10^{-10} mol/L Ang II and the inhibition by 10^{-6} mol/L Ang II, consistent with the AT_1-mediatd biphasic regulation of NBC1. On the other hand, the MEK inhibitor PD9805 abolished only the stimulatory effect of Ang II, leaving the inhibitory effect of Ang II unaffected. These results indicate that the ERK pathway mediates only the stimulatory effect of Ang II in intact proximal tubules [12]. Further analysis showed that the inhibitory effect of Ang II is independent of the ERK pathway, but dependent on the group IVA cytosolic PLA_2 (cPLA2α) activity. These observations indicate that Ang II activates ERK and cPLA2α with different concentration dependency *via* AT_1, and that the balance between ERK and cPLA2α activities determines the final responses to Ang II in intact proximal tubules [12]. It remains to be determined whether the Pyk2/Src coupling, as shown in OK cells [61], is also responsible for the Ang II-induced ERK activation in intact proximal tubules.

ROLES OF ARACHIDONIC ACID METABOLITES IN Ang II ACTIONS

Ang II activates $cPLA_2$ in both VSMCs and renal proximal tubules. $cPLA_2$ preferentially catalyzes the hydrolysis of sn-2 position of glycerophospholipids to release arachidonic acid, which in turn is metabolized to prostaglandins by the cyclooxgenase pathway and to leukotriens by the 5-lipoxygenase pathway [63]. Renal proximal tubules, on the other hand, abundantly express the cytochrome P450 epoxygenase [64], and the arachidonic acid metabolites through this pathway, mainly 5,6- epoxyeicosatrienoic acid (EET), are involved in the inhibitory effect of Ang II [12, 48, 49, 65]. Interestingly, arachidonic acid metabolites seem to play quite contrasting roles in the Ang II-induced ERK activation in VSMCs and renal proximal tubules.

In VSMCs, Muthalif and colleagues identified a key role of hydroxyeicosatetraenoic acid (HETE) in the Ang II-mediated ERK activation [10]. They showed that the stimulation of $cPLA_2$ by Ang II or norepinephrine facilitates arachidonic acid release. P450 metabolites of arachidonic acid, such as 12,15-HETE or 20-HETE, activate ERK by the Ras/Raf/MEK pathway. Activation of MEK/ERK in turn amplifies $cPLA_2$ activity and further releases arachidonic acid. Thus, the $cPLA_2$/P450/HETE pathway and the ERK cascade constitute a positive feedback loop in VSMCs (Fig. (**1**)). This model is also consistent with a vasoconstrictive effect of 20-HETE, which has been attributed to the inhibitory effect on K^+ channel activities [66-68].

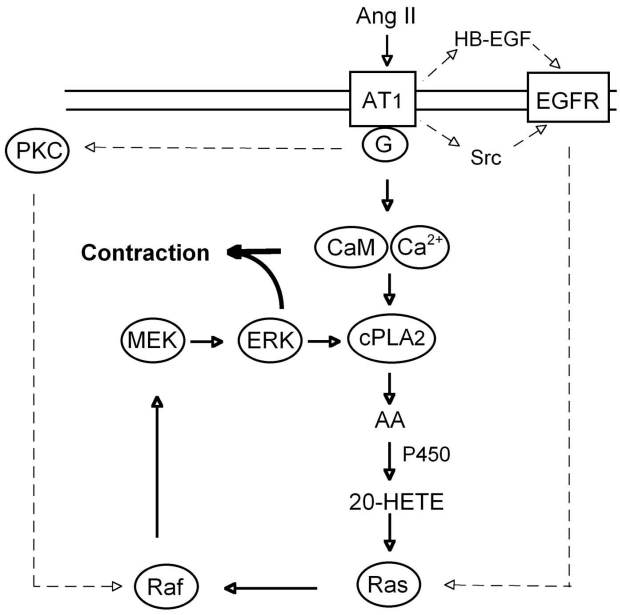

Fig. (1). The role of MEK/ERK pathway in Ang II actions on vascular smooth muscle contraction. In this model, Ca^{2+} ions bind to calmodulin (CaM), which not only facilitates contraction but also activates $cPLA_2$. Arachidonic acid (AA), released by $cPLA_2$, is then metabolized by P450, and metabolites such as 20-HETE activate ERK by the Ras/Raf/MEK pathway through a positive feedback mechanism. While EGFR transactivation either through HB-EGF or Src may activate the Ras/Raf/MEK pathway, PKC may activate the Raf/MEK pathway. The ERK activation also contributes to contraction. G, G protein

By sharp contrast, the cPLA$_2\alpha$/P450 pathway might work to prevent the ERK activation by Ang II in proximal tubules. Thus, 10^{-10} mol/L Ang II significantly activated ERK, but high concentrations of Ang II (> 10^{-8} mol/L) failed to activate ERK in kidney cortex obtained from wild-type mice. When the cPLA$_2\alpha$ activity was abrogated by pharmacological means or genetic knockout, however, high concentrations of Ang II were able to activate ERK [12]. These observations indicate that the cPLA$_2\alpha$/P450/EET pathway rather works as a negative feedback loop on the ERK cascade in intact renal proximal tubules (Fig. (**2**)). This finding is quite surprising, because P450 metabolites are reported to activate ERK in a number of different types of cells such as endothelial cells, arterial smooth muscle cells, glomerular mesangial cells, and renal tubular epithelial cells [69, 70]. However, the further analysis in intact proximal tubules revealed that Ang II failed to activate ERK in the presence of arachidonic acid or 5,6-EET, supporting the negative effect of arachidonic acid metabolites on the ERK pathway (Li, Y and Seki G, unpublished observation). Moreover, the negative effect of EET on ERK activation is consistent with a protective role of EET in Ang II-induced renal injury [71].

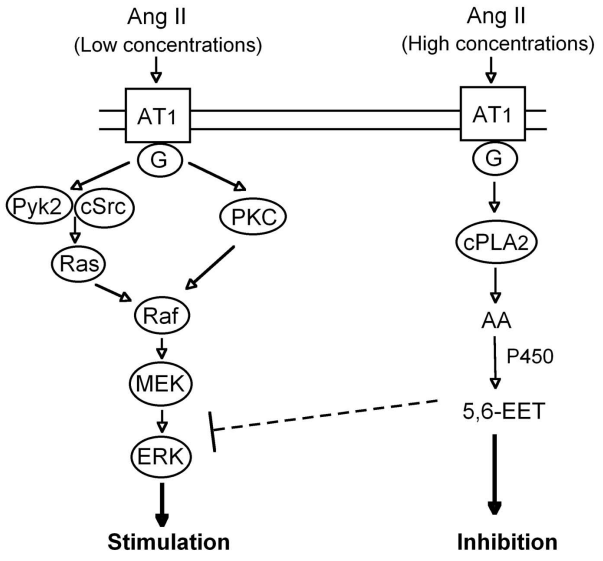

Fig. (2). The role of MEK/ERK pathway in Ang II actions on renal proximal transport.

In this model, AT_1 mediates both stimulatory and inhibitory effects of Ang II. The activation of Raf/MEK/ERK pathway, which is mediated by either Pyk2/cSrc coupling or PKC, is responsible for the stimulatory effect of Ang II. On the other hand, the activation of cPLA$_2$ by higher concentrations of Ang II is responsible for the inhibitory effect. Furthermore, arachidonic acid metabolites such as 5,6-EET may have a negative feedback effect on the MEK/ERK pathway.

At present the exact molecular mechanism underlying the contrasting effects of cPLA$_2$/P450 pathway on the ERK cascade in vascular smooth muscle cells and renal proximal tubules remains unknown. The P450-eicosanoids are known to activate a variety of intracellular signaling pathways, which may be mediated by a putative cell surface receptor or direct intracellular interaction [70]. It is tempting to speculate, however, that renal proximal tubules may have to develop the unique mechanism to attenuate excessive actions of Ang II. Indeed, *in situ* proximal tubular fluid is reported to contain markedly high concentrations of Ang II [44], and uncontrolled Ang II actions may result in unwarranted sequences such as cell hypertrophy or cell damage [14, 71].

OTHER SIGNALING PATHWAYS

Recently other signaling pathway mediating the Ang II-induced ERK activation in vascular tissues have been reported. For example, Min *et al.* reported that aldosterone and Ang II synergistically increase the ERK activation in VSMCs [72]. Chai *et al.* reported that aldosterone potentiates the vasoconstrictor effect of Ang II in coronary arteries [73]. Interestingly, both nongenomic and genomic actions of aldosterone seem to be involved in these interactions, which support the benefit of combination therapy with blockade of aldosterone and AT_1 receptors in the treatment of hypertension and heart-failure [74].

Another interesting observation is the involvement of small G protein Rho in vascular effects of Ang II. Rho has been shown to participate in the formation of focal adhesions and actin stress fibers, as well as in mediating the redistribution of cytoskeletal components [75]. In cardiac myocytes, Aikawa *et al.* reported that Rho-kinase is involved in the stretch-induced ERK activation, but not in the Ang II-induced ERK activation [76]. In mesenteric arteries, on the other hand, Matrougui *et al.* reported that Rho-kinase is involved in the ERK activation and the contraction induced by Ang II [77], suggesting that Rho might be a future therapeutic target.

CONCLUDING REMARKS

This review, which focuses on the mechanism of ERK activation by Ang II, reveals that the cPLA$_2$/P450 pathway has the positive feedback effect in vascular smooth muscle, but has the negative feedback effect in renal proximal tubules. Interestingly, several lines of evidence suggest that the alteration of P450 activities is associated with hypertension in both rodents and human [78-80]. For example, several members of EET are known to have antihypertensive properties, which have been attributed to the effect on vascular tone [70, 81]. However, the negative effect on ERK cascade in renal proximal tubules could be also involved in the antihypertensive properties of EET, since the ERK activation is coupled to the stimulation of proximal transport [12]. For the better understanding of multiple cardiovascular consequences of RAS activation, future studies are warranted aiming to clarify the different effects of arachidonic acid metabolites on ERK-dependent pathways in vascular and renal tissues.

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