Angiotensin II signal transduction through the AT₁ receptor: novel insights into mechanisms and pathophysiology

Sadaharu HIGUCHI∗†, Haruhiko OHTSU∗†, Hiroyuki SUZUKI*, Heigoro SHIRAI*, Gerald D. FRANK† and Satoru EGUCHI*

ABSTRACT

The intracellular signal transduction of AngII (angiotensin II) has been implicated in cardiovascular diseases, such as hypertension, atherosclerosis and restenosis after injury. AT₁ receptor (AngII type-1 receptor), a G-protein-coupled receptor, mediates most of the physiological and pathophysiological actions of AngII, and this receptor is predominantly expressed in cardiovascular cells, such as VSMCs (vascular smooth muscle cells). AngII activates various signalling molecules, including G-protein-derived second messengers, protein kinases and small G-proteins (Ras, Rho, Rac etc), through the AT₁ receptor leading to vascular remodelling. Growth factor receptors, such as EGFR (epidermal growth factor receptor), have been demonstrated to be 'trans'-activated by the AT₁ receptor in VSMCs to mediate growth and migration. Rho and its effector Rho-kinase/ROCK are also implicated in the pathological cellular actions of AngII in VSMCs. Less is known about the endothelial AngII signalling; however, recent studies suggest the endothelial AngII signalling positively, as well as negatively, regulates the NO (nitric oxide) signalling pathway and, thereby, modulates endothelial dysfunction. Moreover, selective AT₁-receptor-interacting proteins have recently been identified that potentially regulate AngII signal transduction and their pathogenic functions in the target organs. In this review, we focus our discussion on the recent findings and concepts that suggest the existence of the above-mentioned novel signalling mechanisms whereby AngII mediates the formation of cardiovascular diseases.

Key words: angiotensin II, angiotensin II type 1 receptor (AT₁ receptor), cardiovascular disease, signalling mechanism, vascular smooth muscle cell.

Abbreviations: ADAM, a disintegrin and metalloproteinase; AngII, angiotensin II; ARAP1, type 1 AngII-receptor-associated protein 1; AT₁ receptor, AngII type-1 receptor; AT₂ receptor, AngII type-2 receptor; ATRAP, AT₁-receptor-associated protein; dn, dominant-negative; EC, endothelial cell; EGF, epidermal growth factor; EGFR, EGF receptor; eNOS, endothelial NO synthase; EP24.15, thimet oligopeptidase; ERK, extracellular-signal-regulated kinase; GEF, guanine nucleotide-exchange factor; GLP, GEF-like protein; GPCR, G-protein-coupled receptor; HB-EGF, heparin-binding EGF; HUVEC, human umbilical vein EC; IGF-1R, insulin-like growth factor-1 receptor; IRS-1, insulin receptor substrate-1; JAK2, Janus kinase 2; JNK, c-Jun N terminal kinase; MAPK, mitogen-activated protein kinase; MYPT, myosin phosphatase targeting subunit; NF-κB, nuclear factor κB; 3NT, 3-nitrotyrosine; p70S6K, p70 S6 kinase; PARP, poly(ADP-ribose) polymerase; PDGFR, platelet-derived growth factor receptor; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PLC, phospholipase C; PYK2, proline-rich tyrosine kinase 2; ROCK, Rho-kinase; ROS, reactive oxygen species; SIP, sphingosine-1-phosphate; STAT, signal transducer and activator of transcription; VSMC, vascular smooth muscle cell.

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INTRODUCTION

AngII (angiotensin II), the major bioactive peptide of the renin–angiotensin system, plays a critical role in controlling cardiovascular homeostasis. It is also strongly implicated in various cardiovascular diseases, such as hypertension, atherosclerosis, restenosis after angioplasty and heart failure. However, the literature regarding the mechanistic insights by which AngII contributes to each of the pathophysiology of these cardiovascular diseases remains obscure. This justifies the substantial amount of research ongoing towards understanding the signal transduction network of AngII within its target organs [1, 2]. There are at least two seven transmembrane GPCRs (G-protein-coupled receptors) known to mediate AngII function, the AT1 receptor (AngII type-1 receptor) and AT2 receptor (AngII type-2 receptor). The AT1 receptor has been shown to mediate most of the physiological and pathophysiological actions of AngII, and this subtype is predominantly expressed in cardiovascular cells, such as VSMCs (vascular smooth muscle cells). Through this receptor, AngII activates a number of cytoplasmic signalling pathways, which may contribute to vascular remodelling, inducing hypertrophy, hyperplasia and migration of VSMCs. The AT1 receptor interacts with multiple heterotrimeric G-proteins, including Gq/11, G12, G13, and G11, and produces second messengers, such as inositol trisphosphate, diacylglycerol and ROS (reactive oxygen species). It also activates various intracellular protein kinases, such as receptor and non-receptor tyrosine kinases and serine/threonine kinases, the MAPK (mitogen-activated protein kinase) family [ERK (extracellular-signal-regulated kinase), JNK (c-Jun N terminal kinase) and p38MAPK], p70S6K (p70 S6 kinase), Akt/PKB (protein kinase B) and various PKC (protein kinase C) isoforms [3–6].

Besides protein kinases, it has been recognized that small GTP-binding proteins (G-proteins), such as Ras, Rho and Rac, are activated by AngII through the AT1 receptor [7]. These small G-proteins appear to play important roles in mediating cardiovascular remodelling induced by AngII. Ras activation induced by AngII causes vascular hypertrophy and hyperplasia. AngII mainly activates Ras through the ‘transactivation’ of EGFR [EGF (epidermal growth factor) receptor]/ErbB1 with recruitment of a Ras GEF (guanine-nucleotide-exchange factor), Sos, via adaptor proteins Shc and Grb2, and subsequently induces the Raf/ERK and PI3K (phosphoinositide 3-kinase)/Akt pathways [7]. Moreover, recent findings have demonstrated the upstream mechanisms as well as downstream significances of growth-factor-receptor transactivation by AngII, which is a major focus of the present review. Rac is an important component of the reduced NADPH oxidase complex to produce ROS by AngII in VSMCs [8]. Rac is also implicated in PAK1 (p21-activated kinase 1) activation by AngII in VSMCs, which subsequently mediates JNK activation and hypertrophy [9, 10]. Rho has been implicated in the Ca2+ sensitization of smooth muscle contraction, whereas recent accumulating evidence further suggest that the Rho/ROCK (Rho-kinase) pathway is critical for vascular remodelling induced by AngII [7].

Endothelial dysfunction is an early event in the pathogenesis of atherosclerosis and a feature of the insulin-resistant condition, including Type 2 diabetes, obesity and hypertension [11, 12]. Not much is known about AngII signalling in ECs (endothelial cells); however, recent evidence suggests that AT1 receptor signalling in ECs induce endothelial dysfunction, possibly through an alternative of NO (nitric oxide) function and induction of vascular insulin resistance [13]. Finally, the C-terminal cytoplasmic domain of the AT1 receptor appears to associate with novel specific protein members with signal transduction properties. These unique AT1-receptor-binding proteins may play an important role in AngII signal transduction pathways leading to cardiovascular remodelling.

On the basis of the above background information, in this review we will focus our discussion on recent findings that suggest the existence of novel signalling concepts of the AT1 receptor by which AngII mediates vascular and endothelial dysfunction, including the growth-factor-receptor transactivation, Rho/ROCK activation, endothelial signal cross-talk and AT1-receptor-binding proteins. Recent review articles focusing on other subjects of AngII signal transduction should also be referenced along with this article, such as those describing the general vascular signals including ROS [2], the relationship with hypertension [14], the link to kidney diseases [15], caveolin-dependent signalling [16], arrestin-dependent signalling [17] and G-protein-independent AngII signalling [18]. In addition to the vasculature, the AT1 receptor is expressed in many other tissues, including kidney, heart, adrenal gland, brain, lung and adipose tissues [19]. Although it may be outside of the scope of this review, it should be noted that some of the functions of AT1 on the cardiovascular system are mediated through other organs, such as through the kidney [20, 21] or brain [22], and not by direct action on the vasculature. Therefore tissue-selective signal transduction studies of the AT1 receptor beyond the cardiovascular system need to be developed further.

RECEPTOR TRANSACTIVATION CASCADES

The significance of the EGFR/ErbB1 transactivation pathway induced by AngII has been well documented in cultured VSMCs [4]. In addition, AngII induces ErbB2/HER2, PDGFR (platelet-derived growth factor receptor) and IGF-1R (insulin-like growth factor-1 receptor) transactivation, which may also contribute to vascular
dysfunction [23–25]. In this section, we will discuss the signal transduction mechanism of receptor tyrosine kinase transactivation by AngII in relation to cardiovascular remodelling.

EGFR transactivation induced by AngII

Previously, we have shown that a tyrosine kinase that exists downstream of PLC (phospholipase C)/Ca\(^{2+}\) mediates ERK1/2 activation by AngII in VSMCs [26]. Subsequently, we identified the tyrosine kinase as EGFR/ErbB1 [27]. Similar to EGF stimulation, AngII rapidly transactivates the EGFR leading to activation of the Ras/ERK cascade. EGFR transactivation by AngII is also required for activation of Akt/PKB, p70S6K and p38MAPK, induction of c-fos and subsequent growth and migration of VSMCs [6]. In addition, there are interesting reports that EGFR transactivation induced by AngII leads to increases in intracellular Ca\(^{2+}\) [28] and ROS [29].

The AngII-induced EGFR transactivation seems to be Ca\(^{2+}\)-sensitive or -insensitive [27,30,31], requires ROS and an upstream kinase, such as c-Src or c-Abl [29,32–34], and involves a metalloprotease-dependent production of a EGFR ligand, such as HB-EGF (heparin-binding EGF), depending on the cell types [35–37]. HB-EGF shedding and subsequent EGFR transactivation induced by AngII appear to be mediated by ADAM17 (a disintegrin and metalloproteinase 17) in ACHN tumour cells [38] and COS7 cells [39]. Recently, we also identified ADAM17 to mediate HB-EGF-dependent EGFR transactivation in VSMCs [40]. Alternatively, other mechanisms participate in AngII-induced EGFR transactivation possibly through an intracellular-signal-dependent cascade [16,29,32]. In this regard, it was reported that phosphorylation of the AT\(_1\) receptor at Tyr\(^{319}\) is required for EGFR transactivation by promoting a recruitment of EGFR [41]. This mechanism appears to be involved in cardiac hypertrophy induced by AngII [42].

Although the possible presence of G\(_q\)-independent EGFR transactivation has been reported [43,44], HB-EGF shedding through ADAM17 induced by AngII requires G\(_q\) activation, since overexpression of a G\(_q\)-inhibitory mini-gene blocked HB-EGF shedding and EGFR transactivation induced by AngII. Also, HB-EGF shedding was not induced by an AT\(_1\) receptor mutant lacking a G\(_q\) coupling [39]. AngII-induced HB-EGF shedding through ADAM17 thus requires second messengers, such as Ca\(^{2+}\) and ROS [39]. In addition to these signalling molecules, ADAM-interacting proteins appear to be involved in EGFR transactivation induced by AngII [10]. Several distinct ADAM-interacting proteins have been identified that include kinases, adaptors or substrates [45]. One of those proteins, Eve-1, associates with ADAMs, including ADAM17, through its SH3 domain and is required for HB-EGF shedding induced by AngII in HT1080 cells [46]. An adaptor protein, PACSIN3, also interacts with ADAMs through its SH3 domain. PACSIN3 appears to be required for HB-EGF shedding induced by PMA and, in part, by AngII in HT1080 cells [47].

The phosphorylation of ADAMs may be an additional mechanism by which AngII activates them. In response to PMA, ERK phosphorylates and activates ADAM17 via Thr\(^{735}\) phosphorylation in CHO (Chinese-hamster ovary) cells [48]. Similarly, p38MAPK appears to exist upstream of ADAMs, leading to HB-EGF shedding and EGFR transactivation induced by environmental stress, including ROS [49]. However, we have shown that ERK and p38MAPK exist downstream of EGFR transactivation induced by AngII in VSMCs [35]. Interestingly, it was reported recently that PI3K activation by c-Src induces ADAM17 phosphorylation through PDK1 (phosphoinositide-dependent kinase 1), leading to EGFR transactivation through amphiregulin shedding in squamous carcinoma cells [50]. Alternatively, upstream tyrosine kinases (c-Src or Abl) might phosphorylate ADAMs in response to AngII, thus leading to EGFR transactivation (Figure 1).

AngII-induced EGFR transactivation regulates VSMC function, such as migration and hypertrophy. We found that metalloprotease inhibition by a pharmacological inhibitor not only attenuated the ERK activation by AngII, but also blocked growth and migration of VSMCs stimulated by AngII [51]. Furthermore, we have recently reported that dn (dominant-negative) ADAM17 markedly inhibited hypertrophy of VSMCs stimulated by AngII [40]. In addition, it has been shown that AngII induces renal deterioration through EGFR transactivation mediated through ADAM17 [52]. Taken together, these data suggest that HB-EGF shedding by ADAM17 and subsequent EGFR transactivation play critical roles in cardiovascular remodelling induced by AngII.

PDGFR transactivation

Transactivation of PDGFR can be induced through the AT\(_1\) receptor in some cells (including VSMCs [53]) and tissues that might have important functions, such as cell growth and migration [54,55]. Also, PDGFR transactivation has been reported to mediate AngII-induced ERK activation in mesangial cells [56]. In the Ren2 rat, a model of AngII-driven hypertension and end-organ damage despite continuous hypertension and left ventricular hypertrophy, cardiac dysfunction was attenuated by a PDGFR kinase inhibitor, imatinib. This tissue-protective effect of the PDGFR inhibitor was associated with decreased transactivation of PDGFR-\(\beta\), activation of ERK, fibrosis and microvascular hypertrophy induced by AngII [55]. Acute AngII infusion also leads to activation of PDGFR as well as EGFR in the vasculature [57]. Imatinib inhibits AngII-induced PDGFR transactivation and subsequent mesenteric arterial hypertrophy and
matrix expansion in the rats regardless of sustained hypertension, suggesting the role of PDGFR transactivation in mediating vascular remodelling [58].

Little is known regarding the mechanism of the PDGFR transactivation. AngII-induced PDGFR transactivation in VSMCs is ligand-independent, but requires a ROS-sensitive tyrosine kinase distinct from Src or JAK2 (Janus kinase 2) [53]. However, H₂O₂-induced PDGFR transactivation appears to require PYK2 (proline-rich tyrosine kinase 2) and Src as well as PKCδ [59]. Interestingly, it has been recently reported that AngII induces phosphorylation of ectodomain-truncated PDGFR-β at Tyr751 and Tyr1021 and recruits the p85 subunit of PI3K without the enhancement of PDGFR kinase activity in VSMCs [60].

IGF-1R transactivation

The significance of IGF-1R transactivation by AngII has been less clear. It was reported that AngII induced tyrosine phosphorylation of IGF-1R as well as IRS-1 (insulin receptor substrate-1) in VSMCs [25]. A Src-dependent IGF-1R intracellular transactivation pathway induced by AngII has been reported in VSMCs [61]. By using the IGF-1R kinase inhibitor AG1024, it was proposed that IGF-1R transactivation is required for PI3K and p70S6K activation by AngII, but not for the stimulation of ERK [61]. By using the same inhibitor, Touyz et al. [62] have suggested that AngII induces ROS production partially through IGF-1R transactivation, leading to p38MAPK and ERK5 activation in VSMCs. Obviously, further research beyond the application of a pharmacological inhibitor is necessary to conclude the significance of IGF-1R transactivation by AngII in mediating vascular pathophysiology.

THE RHO/ROCK PATHWAY

In addition to its primary role in cytoskeletal reorganization and smooth muscle Ca²⁺ sensitization, Rho has been implicated in cardiovascular remodelling...
associated with hypertension and other cardiovascular diseases [63,64]. In VSMCs, AngII increases GTP-bound RhoA [65] as well as RhoA in the particulate fraction [66]. The AT1 receptor is coupled to G12/13 as well as Gq in VSMCs [67,68], cardiac myocytes [69] and cardiac fibroblasts [70]. Therefore RhoGEFs sensitive to G12/13, such as p115RhoGEF, LARG (leukaemia-associated RhoGEF) or PDZ-RhoGEF, may mediate Rho activation [7,71,72]. Gq may also participate in the Rho activation through p63RhoGEF, a RhoGEF-sensing Gq, but not G12 or G13 [73], or tyrosine phosphorylation of a RhoGEF, Vav, may be involved in RhoA activation by AngII in VSMCs [74]. In addition, β-arrestin 1 as well as Gq are required to activate RhoA by AT1 receptors expressed in HEK293 cells (human embryonic kidney 293 cells) [75].

Recently, we have shown [76] that AngII-induced Rho/ROCK pathway activation requires a tyrosine kinase, PYK2, and its upstream activation by PKCδ in VSMCs, by using a ROCK substrate, MYPT (myosin phosphatase targeting subunit) phosphorylation at Thr696, as a marker of Rho activation. In addition, we found that PYK2 may signal to Rho through phosphorylation and activation of PDZ-RhoGEF, since PDZ-RhoGEF was co-immunoprecipitated with PYK2 and it was tyrosine-phosphorylated upon AngII stimulation [76]. Interestingly, an EGFR kinase inhibitor, AG1478, did not affect MYPT phosphorylation induced by AngII, whereas dnPYK2 did not affect EGFR transactivation induced by AngII. Thus the Rho/ROCK pathway activated by AngII is in parallel with EGFR transactivation pathways in VSMCs (Figure 1).

Rho and ROCK have been implicated in AngII-induced vascular remodelling [7]. Alternatively, JNK has been shown to be indispensable for VSMC migration stimulated by AngII [77]. In this regard, dnRho and a ROCK inhibitor, Y-27632, blocked AngII-induced JNK activation [76]. dnRho, Y-27632 and dnJNK inhibited migration of VSMCs induced by AngII as well. These data suggest that activation of Rho/ROCK is specifically required for AngII-induced JNK activation and subsequent VSMC migration [76]. Interestingly, AngII induces phosphorylation of NF-κB (nuclear factor κB)/RelA at Ser536 through RhoA activation, leading to NF-κB activation and subsequent induction of IL-6 (interleukin-6) in VSMCs [78]. In addition, Y-27632 inhibits both AngII-induced MCP-1 (monocyte chemoattractant protein-1) and PAI-1 (plasminogen-activator inhibitor-1) expression in VSMCs [79,80]. These data suggest that ROCK has a critical role in the progression of vascular inflammation.

Recent studies have demonstrated the participation of the Rho signalling pathway in several cardiovascular pathologies, including hypertension, atherosclerosis and restenosis [63,64]. As expected by the role of Ca2+ sensitization, ROCK is reported to participate in AngII-induced vasoconstriction through the AT1 receptor [81]. Also, it has been demonstrated that activation of the Rho/ROCK pathway by ROS is required for the development of spontaneous tone in aorta from AngII-infused rats [82]. AngII infusion increases the activity of RhoA/ROCK, increases medial thickness and promotes perivascular fibrosis in rat coronary arteries. Although oral treatment of fasudil, which is metabolized to a specific ROCK inhibitor, did not prevent AngII-induced hypertension, these vascular alterations were ameliorated by its treatment [83]. In addition, fasudil inhibited the incidence of abdominal aortic aneurysm induced by AngII infusion in ApoE (apolipoprotein E)-deficient mice. Fasudil attenuated aortic capase 3 activity and DNA fragmentation as well as matrix metalloprotease activity, indicating aortic wall apoptosis and proteolysis was suppressed by a ROCK inhibition [84]. Also, perivascular fibrosis induced by AngII was decreased in ROCK1+/− haploinsufficient mice [85]. Thus, in addition to the EGFR transactivation pathway, the parallel Rho/ROCK pathway appears to be a critical signal transduction pathway induced by the AT1 receptor leading to vascular remodelling.

ENDOTHELIAL SIGNALLING OF THE AT1 RECEPTOR

Although much less is known regarding the endothelial AngII signal transduction and function than those in VSMCs, an increasing body of evidence suggests novel roles of the endothelial AT1 receptor signalling in regulating the balance between NO and ROS in ECs [86]. Dysfunctional endothelium, characterized by less production of NO as well as NO bioavailability, leads to accelerated vasoconstriction, smooth muscle cell proliferation and a prothrombotic state as well as inflammation. A decline in NO availability may be caused by decreased expression of eNOS (endothelial NO synthase), a lack of substrate or cofactors for eNOS, alterations in cellular signalling such that eNOS is not appropriately activated (eNOS uncoupling) and accelerated NO degradation by ROS [87].

In this regard, oxidative stress plays a major role in the pathogenesis of endothelial dysfunction and that AngII/AT1 receptor activation is critical in this process [88]. In fact, AngII inhibition [ACE (angiotensin-converting enzyme) inhibitors or AT1 receptor antagonists], antioxidants or genetic disruption of ROS-producing enzymes have been shown to improve endothelial function in human and animal studies [89–91]. NO reacts with superoxide anion, resulting in the formation of peroxynitrite (ONOO−) [92], which possess a high affinity to nitrate tyrosine residues forming 3NT (3-nitrotyrosine) and toxicity. AngII induces 3NT formation in vascular endothelium and inhibits endothelium-dependent vasorelaxation [93].
Signalling mechanism by which the AT₁ receptor modulates endothelial dysfunction

AngII induces ROS production through NADPH oxidase or eNOS uncoupling which mediates endothelial dysfunction. NO reacts with superoxide anion, resulting in the formation of peroxynitrite (ONOO⁻). Peroxynitrite induces endothelial dysfunction through the production of 3NT or PARP. Alternatively, the AT₁ receptor stimulates endothelial NO production through eNOS Ser¹¹⁷⁹ phosphorylation to protect endothelial function. In addition, AngII induces ICAM-1 and VCAM-1 through NF-κB in ECs, and this cascade involves ROS and p38MAPK activation. BH₄, tetrahydrobiopterin; CaM, calmodulin; DHFR, dihydrofolate reductase; ET-1, endothelin-1.

Stimulation of NO production by AngII through the endothelial AT₁ receptor is also reported in cultured ECs as well as in vivo studies, which is thought to be a vascular protection mechanism [94–97]. It has been demonstrated that endogenous H₂O₂, derived from NADPH oxidase, mediates endothelial NO production by AngII [98]. We have recently reported that AngII-induced NO production requires G_q-dependent eNOS Ser¹¹⁷⁹ phosphorylation and that eNOS gene transfer inhibits AngII-induced VSMCs hypertrophy [95]. Alternatively, AngII stimulates formation of S1P (sphingosine-1-phosphate) via activation of sphingosine kinase, which then activates eNOS through the S1P receptor [99].

On the other hand, eNOS may become uncoupled by AngII infusion, causing superoxide production rather than NO production [94,100]. Also, endothelial NADPH-oxidase-derived H₂O₂ induced by AngII was reported to mediate tetrahydrobiopterin deficiency and uncoupling of eNOS [101]. In addition, AngII has been shown to activate PARP [poly(ADP-ribose) polymerase] via the AT₁ receptor through NADPH in ECs [102]. PARP is an energy-consuming enzyme which transfers ADP ribose units to nuclear proteins. As a result of this process, intracellular NAD⁺ and ATP levels decrease remarkably, resulting in EC dysfunction. On the basis of these findings, the balance between the generation of ROS, NO and peroxynitrite in ECs and VSMCs could be an important determinant of the pathological function of AngII in mediating endothelial dysfunction. In addition, a study has shown that AngII, through the AT₁ receptor, inhibits insulin-induced NO production in HUVECs (human umbilical vein ECs) by increased site-specific serine phosphorylation on IRS-1 [103]. In HUVECs, AngII induces phosphorylation of IRS-1.
Table 1  AT1 receptor C-terminal-tail-specific-associated proteins

<table>
<thead>
<tr>
<th>Protein identified</th>
<th>Screened cDNA library</th>
<th>Size</th>
<th>Tissue distribution</th>
<th>Cellular distribution</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>ATRAP</td>
<td>Yeast two-hybrid mouse kidney</td>
<td>18 kDa</td>
<td>Kidney, heart and testis</td>
<td>Three transmembrane protein</td>
<td>[114]</td>
</tr>
<tr>
<td>ARAP1</td>
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<td>57 kDa</td>
<td>Ubiquitous</td>
<td>Cytosolic protein</td>
<td>[120]</td>
</tr>
<tr>
<td>GLP</td>
<td>Yeast two-hybrid mouse embryo</td>
<td>58 kDa</td>
<td>Kidney, pancreas and heart</td>
<td>Cytosolic protein</td>
<td>[121]</td>
</tr>
<tr>
<td>EP24.15</td>
<td>Yeast two-hybrid embryonic kidney</td>
<td>75 kDa</td>
<td>Brain, pituitary and testis</td>
<td>Cytosolic protein</td>
<td>[123]</td>
</tr>
</tbody>
</table>

Figure 3  Specific AT1 receptor C-terminal tail-associated proteins and their functions

ATRAP acts as a negative regulator of AT1-receptor-induced signal transduction, whereas ARAP1 and GLP appear to be positive regulators of AT1. The function of EP24.15 remains unknown.

at Ser312 and Ser616 by JNK and ERK respectively. Thus inhibition of JNK and ERK activity reversed the negative effects of AngII on insulin-stimulated NO production [103]. Furthermore, AngII induces the expression of ICAM-1 (intercellular adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) through NF-κB in ECs, and this cascade involves ROS and p38MAPK activation [104]. Although the AT2 receptor is not the focus of this review, it should be noted that AT2 receptors expressed in the endothelium may counteract the effects of AT1 to prevent endothelial dysfunction, possibly through NO production [86,105]. eNOS phosphorylation at Ser1179 through AT2 receptors was also reported in aortic ECs [106]. The roles of ROS and protein kinases in mediating endothelial dysfunction induced by AngII are illustrated in Figure 2.

AT1 RECEPTOR C-TERMINAL TAIL-ASSOCIATED PROTEINS

GPCRs interact with different classes of intracellular proteins, including heterotrimeric G-proteins, GRKs (GPCR kinases) and arrestins in general [107,108]. Although the intracellular third loop of the AT1 receptor is a key structural determinant in coupling the receptor to heterotrimeric G-proteins, a number of studies have highlighted the functional importance of the C-terminal cytoplasmic domain in AT1 receptor signalling and desensitization and internalization [6,17,18,109]. The C-terminal cytoplasmic domain of the AT1 receptor has been reported to directly associate with several non-G-protein signalling molecules, such as JAK2 and PLCγ1 [110,111]. The C-terminal tail also provides a binding site to form homo- and hetero-dimers of the AT1 receptor [112,113]. Therefore, in addition to desensitization and internalization, the C-terminal cytoplasmic domain of the AT1 receptor appears to have critical roles in signal transduction and other receptor functions [6], and also suggests a possibility that the C-terminal cytoplasmic domain of the AT1 receptor might interact with previously unrecognized cellular proteins that are unique to the AT1 receptor, which may be a key determinant in the efficacy and/or specificity of receptor function (Table 1).

ATRAP (AT1-receptor-associated protein) was identified as a specific binding protein to the C-terminal cytoplasmic domain of the AT1 receptor by using a
yeast two-hybrid analysis [114]. ATRAP is a three-transmembrane protein and may function as a negative regulator of AT1-receptor-induced signal transduction [115,116]. Overexpression of ATRAP in VSMCs potentiated AT1 receptor internalization upon AngII stimulation [117]. AngII-induced DNA synthesis was markedly inhibited in these VSMCs and was associated with the inhibition of STAT3 (signal transducer and activator of transcription 3) and Akt activation. In cardiac myocytes, overexpression of ATRAP also decreases AT1 receptors on the cell surface, and decreases subsequent p38MAPK activation, c-fos promoter activation and protein synthesis induced by AngII [118]. Although ATRAP transgenic mice had no significant phenotype, neointimal formation after vascular injury was attenuated and this was associated with reduced activity of ERK, STAT1 and STAT3 [119].

Two other AT1 C-terminal tail-associated proteins, ARAP1 (type 1 AngII receptor-associated protein 1) and GLP (GEF-like protein), have been identified by the yeast two-hybrid system [120,121]. ARAP1 appears to bind and promote the AT1 receptor recycling to the plasma membrane. Interestingly, ARAP1 transgenic mice that overexpress ARAP1 specifically in proximal tubules have hypertension [122], suggesting that renal ARAP1 increases blood pressure through enhancement of the intrarenal AT1 signalling. GLP, a cytosolic protein, contains a motif of seven tandem repeats similar to a kind of GEF for the small G-protein Ran [121]. Overexpression of GLP induced hypertrophy in VSMCs and renal proximal tubular cells. The hypertrophic effect is, at least in part, mediated through Akt activation and inhibition of p27kip1 protein expression [121]. Taken together, these data suggest that the AngII-induced hypertension and hypertrophy might be positively regulated by these AT1 receptor C-terminal tail-associated proteins (Figure 3). EP24.15 (thimet oligopeptidase; EC 3.4.24.15) associates with the C-terminal cytoplasmic domain of the AT1 receptor and the B2 bradykinin receptor [123]; however, the exact function of EP24.15 remains unclear.

CONCLUSIONS

As described above, select findings of the AngII/AT1 signal transduction pathway in the cardiovascular system demonstrate progress in this area and are updated in the present review. Each of the AngII signalling components could play a specific role in mediating vascular remodelling or endothelial dysfunction, leading to cardiovascular diseases, such as hypertension, atherosclerosis and restenosis. However, since most of the findings discussed in this review are from experiments at the cellular level, it is still unclear whether these cascades are sufficiently applicable to explain human diseases. Therefore, in order to expand these research areas, the confirmation of the findings by animal models or human samples will definitely be necessary. In addition, further progress on AngII signal transduction research by using genomic and proteomic approaches will help to better understand the mechanism of cardiovascular diseases and its complications.

ACKNOWLEDGMENTS

This work was supported by National Institute of Health Grants HL076770 (S.E.) and HL076575 (G.D.F.), by an American Heart Association Established Investigator Award 074002N (S.E.), and by W. W. Smith Charitable Trust Grant H0605 (S.E.).

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Received 1 December 2006/3 January 2007; accepted 5 January 2007
Published on the Internet 13 March 2007, doi:10.1042/CS20060342