Versatility of the Angiotensin II Type 1 Receptor

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This issue of Circulation Research includes 3 interesting studies that represent new concepts of AT1 receptor–mediated cell signaling in the cardiovascular system, which is currently of intense interest. The AT1 receptor mediates many important cardiovascular responses, including vasoconstriction, vascular and cardiac remodeling (cell proliferation, hypertrophy, and production of extracellular matrix), and cell survival/cell death. The AT1 receptor belongs to the seven membrane–spanning GPCR family and typically activates PLC through the heterotrimeric Gq protein, causing production of inositol trisphosphate and diacylglycerol. Besides this classical GPCR-Gq-PLC pathway, recent studies indicate that Ang II activates both nonreceptor-type and receptor-type tyrosine kinases, which are typically activated by cytokine and EGFR stimulation. Activation of tyrosine kinases by Ang II is of great interest for a variety of reasons. First, tyrosine kinase–dependent signaling pathways mediate major growth effects of Ang II in cardiovascular systems. Sayeski et al2 reports that a 130-kDa protein, which is tyrosine-phosphorylated by Ang II, is identified as p130Cas and that it potentially works as an effector of Src and PKC in VSMCs. Second, since the AT1 receptor possesses neither intrinsic protein tyrosine kinase activities nor known physical association with tyrosine kinases except interaction with JAK2,3 the linkage between the AT1 receptor and the tyrosine kinases was unexpected. Murasawa et al4 reports that Ang II transactivates the EGFR, which in turn mediates DNA synthesis in cardiac fibroblasts. Third, tyrosine kinases mediate activation of small GTP binding proteins (the Ras and Rho family) and downstream MAP kinases. Schmitz et al5 reports that Ang II activates the αPAK-JNK pathway via a tyrosine kinase–dependent mechanism in VSMCs. Since a comprehensive review on tyrosine kinase activation by Ang II has appeared recently,6,7 the focus of this editorial is to highlight findings of the most recent literature, including both cardiovascular and noncardiovascular systems, and the future direction of those new concepts regarding AT1 receptor cell signaling.

Nonreceptor-Type Tyrosine Kinases

Since several groups have reported that Ang II increases the phosphoryrosine content of cellular proteins in liver GN4 cells, VSMCs, and cardiac myocytes/fibroblasts, many of these phosphoryrosine-containing proteins and the tyrosine kinases, which are responsible for the tyrosine phosphorylation, have been identified (reviewed in Reference 1). The phosphoryrosine-containing proteins identified thus far include protein kinases/phosphatases (Jak2, Tyk2, FAK, PYK2, Src, ERK, and PTP-1D), enzymes/regulatory proteins (PLC-γ1 and IRS-1), adapter proteins (Shc and paxillin), and transcription factors (Stat1, Stat3, and Stat5).6

Sayeski et al2 found that another protein, p130Cas, is tyrosine-phosphorylated by Ang II in VSMCs. It has been recently shown that p130Cas mediates FAK-promoted cell migration on fibronectin in CHO-K1 cells.7 Interestingly, p130Cas physically interacts not only with Src and pp120 but also with PKCα in VSMCs. Sayeski et al have proposed that p130Cas works as a docking site, which attracts molecules associated with the 3 separate important signaling pathways (namely, Src, PKC, and focal adhesion–related signaling pathways) into one place. The physical interaction between p130Cas and these individual signaling molecules apparently takes place with different kinetics. These spatial and temporal regulations of protein-protein interactions will allow the AT1 receptor to control cellular events with more precision.

Ang II activates Src-family tyrosine kinases in cardiac myocytes and VSMCs.8,9 Activation of Src-family tyrosine kinases by Ang II mediates tyrosine phosphorylation of many proteins, including phospholipase Cγ1, Shc, pp97, pp120, and p130Cas, and activation of the downstream signaling molecules, such as Ras and ERKs.10 The mechanism of Src activation by AT1 receptor stimulation has not been fully elucidated. In VSMCs, activation of PLC and the subsequent production of inositol trisphosphate and diacylglycerol depend on Src, since PLC-γ1 is the predominant isoform of PLC in this cell type.11 Tyrosine kinase may control the function of heterotrimeric Gq protein, since tyrosine phosphorylation of the C-terminal end amino acid sequence in Gqα has been shown to be essential for the function of Gqα.12 These results raise an interesting question: Is Src activated by a mechanism independent of (or rather upstream from) the Gq-PLCβ pathway? The definitive answer to this question does not seem to have been obtained as of yet. Although JAK2 is activated by Ang II through direct association with the C-terminal cytoplasmic domain of the AT1 receptor, such direct association between Src and the AT1 receptor is unlikely.1 Multiple mechanisms have been hypothesized for Src activation by other growth factors. These include involvement of receptor-type or nonreceptor-type tyrosine kinases,13 tyrosine phosphatases,14 the Gβγ subunit,15 and high-affinity SH2/SH3 domain–containing proteins.16 It remains to be seen whether or not these molecules contribute to Ang II–induced Src activation.

Besides Src, FAK and PYK2 (also known as Ca2+-dependent tyrosine kinase) are also activated by Ang II.16 The
Selected Abbreviations and Acronyms

Ang II = angiotensin II  
AT1 = Ang II type 1  
EGF = epidermal growth factor  
EGFR = EGF receptor  
ERK = extracellular signal–regulated kinase  
GPCR = G protein–coupled receptor  
JAK = Janus kinase  
JNK = c-Jun N-terminal kinase  
MAP kinase = mitogen-activated protein kinase  
PAK kinase = p21-activated kinase  
PKC = protein kinase C  
PLCβ = phospholipase Cβ  
VSMC = vascular smooth muscle cell

Ras- and Rho-Family Small GTP Binding Proteins and Downstream MAP Kinases

Small GTP binding proteins, such as those in the Ras and Rho families, are activated by ligands for the tyrosine kinase receptors, the cytokine receptors, and the GPCRs. A growing number of molecules have been identified as effector molecules for each small GTP binding protein (reviewed in Reference 23). Regulation of the MAP kinase cascades is one of the most important functions of the small GTP binding proteins. Ang II activates Ras in both cardiac myocytes and VSMCs. Aoki et al have recently demonstrated that Ang II activates RhoA, which in turn mediates premyofibril formation as well as atrial natriuretic factor expression in cardiac myocytes. Schmitz et al have reported that Ang II activates protein-serine/threonine kinase PAK VSMCs. Since PAK interacts with and is activated by the GTP-bound form of Cdc42 and Rac, the Rho-family small GTP binding proteins, it is likely that Ang II activates Cdc42 and Rac. The kinase domain of the PKA is most closely related to yeast Ste20p, a known regulator of MAP kinase pathways. In fact, Schmitz et al have reported that αPAK mediates Ang II–induced activation of JNK. Activation of each small GTP binding protein and respective downstream MAP kinase cascade seems to be mediated by a distinct tyrosine kinase. Src and Ca2+-dependent tyrosine kinase have been shown to mediate Ang II–induced Ras activation. Interestingly, Schmitz et al have suggested that a tyrosine kinase other than Src is involved in Ang II–induced αPAK activation. The Ca2+-dependent tyrosine kinase activation is correlated with stimulation of the JNK and p70S6K pathways but not with ERK or p90RSK in liver GN4 cells. It has been suggested that tyrophostin-sensitive (unidentified) tyrosine kinases mediate activation of Rho by GPCRs. Although Ras is constitutively in the cell membrane, Rho and Rac are predominantly cytosolic and must translocate to the cell membrane to be activated. Therefore, the distinct subcellular localization seems to be well correlated with the hypothesis that the Ras and Rho families may be regulated by distinct tyrosine kinases.

Direct Association of Signaling Molecules With the AT1 Receptor

The AT1 receptor has been shown to directly associate with intracellular signaling molecules, such as Jak2, PTP-1D, and PLC-γ1. This ligand-dependent interaction requires a YIPP motif in the C-terminal domain of the AT1 receptor.
interaction between the AT₁ receptor and intracellular signaling molecules creates membrane-delimited signal transduction complexes similar to those observed for receptor type tyrosine kinases. The AT₁ receptor associates with a Stat5 transcription factor after Ang II stimulation and apparently forms complexes with JAK2. The putative Stat5 binding motif (YXXL) has been found on intracellular loop 1 and on the carboxyl tail of the AT₁ receptor. Small GTP binding proteins, ARF and RhoA, have been shown to interact with the amino acid sequence containing NPXXY (amino acids 298 to 302) in the seventh transmembrane domain of the AT₁ receptor in an Ang II–dependent manner in rat anterior pituitary cells. This direct interaction between the GPCR and ARF/RhoA seems to enhance coupling between the GPCR and phospholipase D. Interestingly, the NPXXY motif is not found in all Gq-coupled receptors. Conversion of the DPXXY motif in another Gq-coupled gonadotropin-releasing hormone receptor to the NPXXXY motif confers sensitivity to an inhibitor of ARF, suggesting that GPCRs utilize the sequence-specific cell signaling. A similar direct interaction between GPCRs and the signaling molecule has been recently reported in the case of β-adrenergic receptor and the Na+/H+ exchanger. The functional role of the direct interaction between the GPCR and signaling molecules, especially whether the interaction is the cause or the result of activation of the signaling molecules, remains to be determined. Nonetheless, these receptor sequence–specific cell signals initiated by GPCR stimulation confer more versatility to the GPCRs.

Unsolved Questions and Future Directions

The AT₁ receptor signaling demonstrates diverse cell-type specificity. Li et al. reported an interesting observation that PKC activation normally suppresses the tyrosine kinase (EGFR)-Ras-ERK pathway. However, once PKC is inactivated, this tyrosine kinase–dependent pathway completely compensates for ERK activation in liver GN4 cells. If this observation is applicable to other cell types, the PKC dependence of ERK activation would be determined by the strength of the tyrosine kinase activities of the given cell types.

Another important issue is the unique Ang II–dependent cell signaling among Gq agonists. In cardiac myocytes, many Gq-agonists, including Ang II, phenylephrine, and endothelin-1, are known to induce cardiac hypertrophy. These agonists seem to activate a similar set of signaling molecules. However, the profile of the signaling molecules activated by hypertrophic agonists has not been elucidated in detail. A recent report suggests that phenylephrine activates p38, resulting in an impressive hypertrophic response in cardiac myocytes. It would be interesting to compare activation of p38 among hypertrophic agonists. If these receptors use sequence-specific signaling mechanisms as described above, sequence comparison among GPCRs would provide pertinent information.

Recent progress in the Ang II signaling field has made the linkage between AT₁ receptor and tyrosine kinase more clear and more significant compared with that a few years ago. We now understand in part why Ang II activates an array of signaling molecules as well as how Ang II can stimulate cell growth responses of the cardiovascular system almost as potently as agonists for the receptor-type tyrosine kinases do in other cell types. However, the mechanisms of many events proximal to the AT₁ receptor, such as tyrosine kinase activation, possible G protein–independent signaling mechanisms, and small GTP binding protein activation, remain unclear. Further investigation, including the structure-function analysis of the AT₁ receptor and identification of the receptor–associating protein, will be required to address such questions.

References


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