Studied of the large families of seven transmembrane receptors have focused on their signal transduction pathways initiated by their interaction with GTP-activated proteins. Angiotensin II (Ang II) mediates most of its well-known cardiovascular effects in a conventional manner through its type 1 (AT1) receptor acting on heterotrimeric G proteins. The affinity of the type 2 (AT2) receptor specific for the G protein Goi fails in large part to explain its functional activity. It has generally opposing effects to AT1. Although it is abundant in the fetus, its distribution is limited in the adult but is induced again under pathological conditions. Some of its important modulator signaling involves direct activation of the tyrosine phosphatase SHP-1 independent of heterotrimeric G protein signaling.1–4 In addition it is atypical in its general failure, unlike AT1, to quickly desensitize and down-regulate with ligand binding. The following overview looks at some AT2 functions and responses that are not mediated by an interaction with heterotrimeric G proteins. Recent articles reveal a new regulatory mechanism of AT2 signaling.

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Heterotrimeric G proteins and arrestins interact specifically and in primary fashion with the intracellular loop 3 of 7 transmembrane receptors. Interaction of the receptors with adapter and scaffolding proteins is important for their cellular sorting and targeting to the cell membrane. The C-terminal tail of the 7 transmembrane receptors has the primary binding sites for these adaptor and scaffolding proteins.5–8 Yeast-two-hybrid screening has been frequently used to identify potential binding proteins at the C-terminal tail of the receptor. In an earlier study in 2004, Nouet et al9 used the C-terminal tail of the AT2 receptor with a mouse fetal cDNA library in a yeast-two-hybrid screening. They isolated a family of 4 binding proteins that they named ATIP. The proteins were able to dimerize and possess a common domain with a large coiled-coil region that binds to AT2. Ectopic expression of ATIP in AT2-expressing cells led to effects similar to that of the AT2 receptor, such as inhibition of EGF signaling. It required the presence of the AT2 receptor for its inhibitory action, which was further augmented by the addition of Ang II. The distribution of ATIP was widespread in many more tissues than those expressing AT2.

In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Wruck et al10 performed a similar study using yeast-two-hybrid screening with 51 amino acids of the AT2 C-terminal tail and a mouse 11-day embryo cDNA library. Four binding protein isoforms designated ATBP were isolated. The short ATBP50 isoform was seen in all tissues tested. It was strongly expressed in uterus and adrenal tissues that express AT2 strongly. In AT2 null mice the ATBP expression was much reduced. Real-time polymerase chain reaction (PCR) analysis of uterus in AT2-null mice showed ATBP mRNA reduced to <10% of its expression in wild-type mice. The 400-aa residue C-terminal of all the isoforms was identical. There was an α-helical coiled-coil motif in the center of this sequence. Using a Golgi marker, the ATBP and marker colocalized in the perinuclear region. The Golgi localization was dependent on the noncoiled C-terminus. Functionally, the binding protein traffics the receptor from the Golgi to the cell surface. The protein appears to be required for cell surface expression of the AT2 receptor, and AT2 function is dependent on the binding protein. This protein is identical to the ATIP protein isolated by Nouet et al. It is also identical to a ubiquitously expressed tumor suppressor protein localized in mitochondria.11 This indicates that this protein has functions unrelated to AT2 in addition to a chaperon function for AT2 signaling.

Senbonzactu et al12 reported a complex unorthodox signaling pathway for the AT2 receptor in the heart. They initially found that their AT2-null mice did not develop the expected cardiac hypertrophy with pressure overload. In their further investigation they used a heart cDNA library with the AT2 C-terminal tail as bait in a yeast-two-hybrid screening. They identified the transcription factor PLZF as an AT2 C-terminal tail-binding partner. This transcription factor has its highest level of expression in the heart. Ang II stimulation of the heart AT2 receptor resulted in tyrosine kinase phosphorylation and activation of PLZF at its carboxyl terminal region. The PLZF translocated from the cytosol to the plasma membrane, colocalizing with the AT2 receptor. Both molecules subsequently translocated from the plasma membrane. Unlike Ang II mediated AT2 endocytosis, this was a relatively slow (~60 minutes) but clearly visualized endocytic process. β-Arrestin is not involved in this endocytosis. The AT2 localized in the perinuclear region, presumably in the endosomal region involved in membrane recycling. The PLZF translocated into the nucleus. PLZF was associated with Epsin 1. PLZF binds to the N terminal portion of Epsin 1, a cytosolic protein involved in clathrin-mediated endocytosis. By shuttling to the nucleus Epsin 1 appears to connect the endocytotic machinery to nuclear function.13 The nuclear PLZF bound to the promoter of p85α, the regulatory unit of P13 kinase, and activated its transcription.
Expression of p85α activated the downstream pathway stimulating protein synthesis. This downstream pathway includes PI3K, AKT, and ribosomal S6 kinase. P85α in addition interacts as a cofactor with ribosomal S6 kinase, further enhancing its activity. These studies indicate a specific contributing rather than antagonistic role of the AT2 receptor in pressure overload–induced cardiac hypertrophy. The greatly enhanced p85 alpha interacts with and is dependent on growth factors such as EGF or IGF 1 for activation of the protein synthesis pathway. p85α also stimulates the GTPase activity of Rab 5 and Rab 4 thereby playing a role in growth factor receptor endocytosis (Rab 5) and growth factor receptor recycling (Rab 4).^{14}

In a recent study by Feng et al.,^{15} Ang II interacting with the AT2 receptor mediated SHP-1 tyrosine phosphatase activation in NIE 115 cells and transfected COS-7 cells. Using mutant receptors they showed that SHP-1 activation is completely independent of G protein activation. An unexpected possibly controversial finding, because in other studies the AT2 receptor third loop does not interact with Gαs, was the constitutive association of the isolated alpha subunit Gαs (not the heterotrimeric G protein) with both AT2 and SHP-1. This linkage appeared essential to the Ang II activation of SHP-1.

References

Beyond the G Protein: The Saga of the Type 2 Angiotensin II Receptor

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