Putting Pressure on the Angiotensin Receptors

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Angiotensins are a family of peptides that are critical players in the control of water and electrolyte balance. Angiotensins mediate their actions via several specific G-protein coupled receptors: AT_1, AT_2 and MAS receptors. Alomone Labs offers a variety of antibodies targeted against these receptors, which are important tools responsible for advancing research in this field. Here, we briefly describe the significant roles and activities of these receptors and their involvement in some cardiovascular and renal diseases.

Angiotensin System

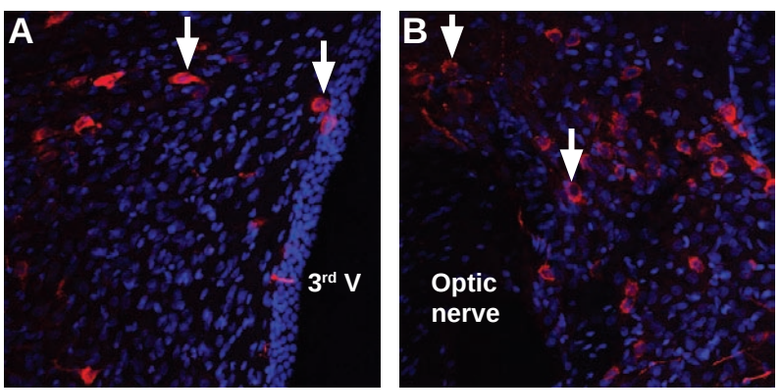
The Renin-Angiotensin System (RAS) or the Renin-Angiotensin-Aldosterone System (RAAS) is a hormonal system that regulates blood presure and water balance and is mainly activated upon loss of blood volume or a drop in blood pressure[3]. Angiotensin (Ang) was discovered in the late 1930s and is a key factor in the RAAS system. This hormone is a derivative of a precursor molecule – angiotensinogen, a 452 amino acid protein, a member of the serpin family, produced as α-2-globulin in the liver. Angiotensinogen is a substrate of the enzyme Renin in the kidney to produce a deca peptide – Ang I that appears to have no biological activity. Ang I is converted to Ang II by Angiotensin-Converting Enzyme (ACE) and this product is the active hormone, which mediates and controls most of the RAAS system effects[3,17]. Ang II is also a precursor for some peptides including the heptapeptide active hormone Angiotensin (1-7) (Ang-(1-7))[24].

Angiotensin II

The biological effects mediated by Ang II are diverse, widespread, and play a critical role in the regulation of the renal and cardiovascular systems, and some activities in the central nervous systems. Circulating levels of Ang II was identified to control blood pressure based on the regulation of salt and water metabolism, vascular smooth muscle cell (VSMC) tone, thirst and sympathetic system outflow. Ang II is well known to cause potent increases in systemic and local blood pressure via its vasoconstrictive effect, to influence renal tubules, to retain Na^+ and water by activating Na^+/H^+ exchangers, and by stimulating aldosterone release from the adrenal gland[11].

Although Ang II was originally discovered as a potent vasoconstrictor, research in the past several decades has provided ample evidence that Ang II modulates cell growth depending on the cell type, subtype of Ang II receptor, and the presence of other hormones, growth factors, and cytokines[7]. Ang II may either stimulate growth (proliferation, hypertrophy) or act as a growth suppressor (apoptosis, antiproliferation with induction of differentiation). Morever,

Expression of Angiotensin II Receptor Type-2 in Rat Brain.



Immunohistochemical staining of rat brain sections using **Anti-Angiotensin Receptor Type-2 (extracellular)** antibody (# AAR-012). A. AT_2 receptor expressing neurons (red) are scattered in the paraventricular nucleus of the hypothalamus (arrows), in the vicinity of the 3rd ventricle (3rd V). B. AT_2 receptor expressing neurons in the supraoptic nucleus (arrows), adjacent to the optic nerve. Nissl is used as the counterstain (blue). Experimental procedure and figure processed at Alomone Labs.

Ang II encourages cell growth in various diseases such as atherosclerosis, vasculitis, hypertension-induced injury, cardiac hypertrophy, compensatory renal hypertrophy, and nephritis[7,11].

Angiotensin II Receptors

With more than two Ang II receptor subtypes, Ang II receptors type 1 and 2 (AT_1 and AT_2, respectively) were initially discovered by a pharmacological approach using various specific Ang II Receptor pharmacological blockers[5]. These drugs antagonize Ang II-induced biological actions, including smooth-muscle contraction, sympathetic pressure mechanisms, and aldosterone release. This paradigm was primarily confirmed by the cloning of AT_1 receptor[15,18] and AT_2 receptor[14]. These findings enabled the understanding of the structure and the function of these receptors. Other poorly characterized subtypes of AT receptor are AT_3 and AT_4 receptors. The AT_4 receptor is activated by the Ang II metabolite Ang IV and may play a role in regulating the CNS extracellular matrix formation[7]. All Ang II receptors have seven transmembrane domains and belong to the superfamily of G-protein coupled receptors (GPCRs).

AT_1 Receptor

Most of the well-known Ang II effects such as blood pressure elevation, vasoconstriction, increase in cardiac contractility, aldosterone release from the adrenal gland and consequently renal Na^+ and water absorption, and facilitation of catecholamine release from nerve endings[21], drinking response and cell proliferation are mediated through the AT_1 receptor. In rat, AT_{1a} and AT_{1b} share 94% homology and have similar pharmacological properties and tissue distribution patterns[21]. AT_1 receptor subtypes are ubiquitously and abundantly distributed in adult tissues, including blood vessel, heart, kidney, adrenal gland, liver, brain, and lung[7].

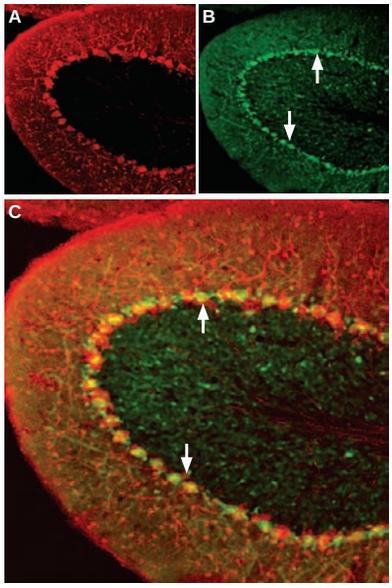
Although the AT_1 receptor has been reported to interact with several G-proteins, its major physiological functions are expressed through $G_{q/11}$-mediated activation. In cardiac fibroblast, AT_1 receptor is coupled to a G_i protein in contrast to AT_1 receptor in cardiac myocytes which couples G_q. Consequently, the downstream signaling differs significantly between these two cell types: In rat cardiac fibroblasts, AT_1 receptor induces ERK tyrosine phosphorylation and cell proliferation. This signaling is also relevant for the expression of collagen and fibronectin during ventricular hypertrophy[7]. In rat cardiac cells, AT_1 receptor induces the activation of phospholipase C β isoforms, and consequently Ca^{2+} release from intracellular stores, thereby inhibiting adenylate cyclase and activating Ca^{2+} influx[7,21].

Many *in vitro* and *in vivo* studies support the notion that Ang II activities (mediated by AT_1 receptor) participate directly in the pathogenesis of various cardiovascular diseases such as left ventricular hypertrophy and renal diseases. Numerous selective and potent nonpeptide AT_1 receptor antagonists have been developed, of which several have been in use clinically for the treatment of hypertension[4,6].
Due to the central role of AT_1 in vasoconstriction, AT_1 receptor antagonists (Type 1 blockers) are effective for the treatment of essential hypertension, by lowering arterial pressure without causing reflex tachycardia. In patients with heart failure AT_1 receptor blockers decrease left ventricular filling pressures and pulmonary arterial pressures and improve cardiac output[6,12]. Also, these blockers have beneficial effects in delaying the onset and the progression of renal disease and diabetic associated hypertension[22].

AT_2 Receptor

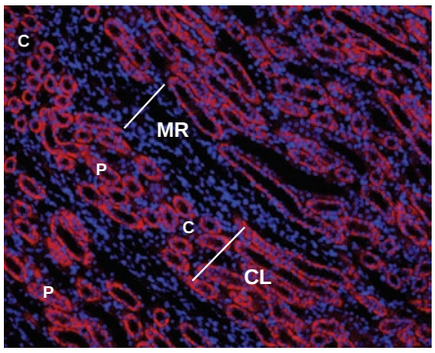
AT_2 receptor is ubiquitously expressed in developing fetal tissues and declines drastically in adult[6]. The expression of the AT_2 receptor was shown to be dependent on growth factors or

Expression of Angiotensin II Receptor Type-1 in Mouse Cerebellum.



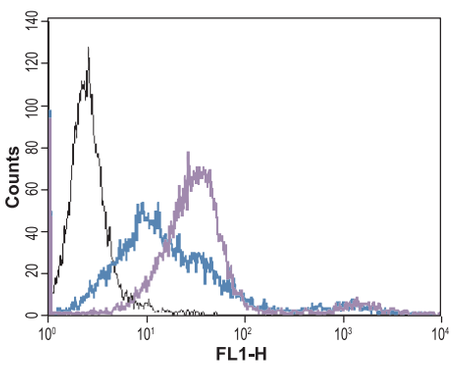
Immunohistochemical staining of mouse cerebellum using **Anti-Angiotensin II Receptor Type-1 (extracellular)** antibody (#AAR-011). AT_1 receptor is present in the Purkinje layer (green). Arrows point at AT_1 receptor immunoreactive cells. Staining of the same section with mouse anti-Parvalbumin (red) reveals partial co-localization (C).
Experimental procedure and figure processed at Alomone Labs.

Expression of Angiotensin II Receptor Type-1 in Rat Kidney.



Immunohistochemical staining of rat kidney paraffin-embedded sections using **Anti-Angiotensin II Receptor Type-1 (extracellular)-ATTO-550** antibody (#AAR-011-AO), (1:50), (red). Staining is specific to the most inner layer of the cortex. Intense staining is present in proximal tubes (P) but not in collecting ducts (C) in the cortical labyrinths (CL). No staining is present both in thin portions of the Loop of Henle or in the collecting ducts in the medullar rays (MR). Nuclei are visualized with Hoechst 33342 (blue).
Experimental procedure and figure processed at Alomone Labs.

Indirect Flow Cytometry Analysis of Live Intact Human Jurkat T-Cell Leukemia Cells.



Jurkat cells + goat-anti-rabbit-FITC.
Jurkat cells + **Anti-Angiotensin II Receptor Type-1 (extracellular)** antibody (#AAR-011), (5 µg) + goat-anti-rabbit-FITC.
Jurkat cells + Anti-Angiotensin II Receptor Type-1 (extracellular) antibody (10 µg) + goat-anti-rabbit-FITC.
Experimental procedure and figure processed at Alomone Labs.

growth states[23]. In mouse 3T3 cells, AT_2 mRNA is expressed when growth is arrested at the confluent state and is decreased in the vigorous growth phase, suggesting a possible role for this receptor in the developing fetus and organ morphogenesis[7]. On the other hand, experiments in AT_2 knockout mice do not demonstrate any siginificant effect on the fetus[10]. In the adult, expression of this receptor is limited mainly to the uterus, ovary, certain brain nuclei, heart, adrenal medulla and its expression is very low in the cardiovascular system. Unlike the AT_1 receptor there is no evidence for subtypes of the AT_2 receptor[23].

In contrast to AT_1, the AT_2 receptor couples to the G_i protein. In various cell lines and vascular smooth muscle cells, AT_2 receptor-activated protein tyrosine phosphatase was shown to inhibit cell growth and induce differentiation or apoptosis[6]. AT_2 receptor was also shown to negatively modulate ERK giving rise to the similar cellular outcomes[13].

In endothelial cells, AT_2 receptor-mediated actions are inhibitory to AT_1 receptor-mediated mitogenic cell growth, demonstrating a contradictory action and indicating a balancing mechanism for Ang II[7].

However, there have been conflicting findings regarding these receptors. *in vivo* studies indicate that the stimulation of the AT_2 receptor increases renal medullary and cortical interstitial cGMP by a bradykinin-dependent mechanism and elevates prostaglandin production, suggesting an important role in renal function, including vasodilatation and blood pressure regulation[6]. In addition, in mice lacking the AT_2 receptor, the drinking response is impaired and locomotion is reduced, and the animals exhibit an increase in vasopressor response to Ang II[13].

Angiotensin (1-7) Mas Receptor

The angiotensin (1-7) Mas receptor was recently identified as a receptor for the biologically active heptapeptide Ang (1-7). This peptide is a metabolite of Ang II produced by ACE2 and its production is directly dependent on Ang II availability[1,17,24].

Considerable interest in Ang-(1-7) and its receptor have been aroused since it became apparent that it can counter balance most of Ang II effects. Thus Ang (1-7) has antidiuretic, vasodilator, and hypotensive effects as well as antiarrhythmic and cardioprotective roles[8]. In addition, activation of Ang-(1-7) Mas receptor reduces vascular growth *in vitro* and *in vivo*[9], inhibits cardiac myocyte growth and mediates inhibition of serum-stimulated MAPK activation in these cells. Moreover, this receptor plays a role in adjusting learning and memory in the brain[20].

Ang-(1-7) Mas receptor was originally described as a proto-oncogene. Evidence indicates that the receptor is coupled to a $G_{q/11}$ protein that activates phospholipase C[16]. It is expressed in several organs including heart, kidney, blood vessels, testis and brain. An intense expression of this receptor was demonstrated in rat heart, and this level of expression is increased in myocytes surrounding the myopathic ischemic zone[2,8].

Alomone Labs offers high quality Angiotensin receptor antibodies for research in this field: **Anti-Angiotensin II Receptor Type-1 (extracellular)** (#AAR-011), **Anti-Angiotensin II Receptor Type-2 (extracellular)** (#AAR-012) and **Anti-Angiotensin-(1-7) Mas Receptor** (#AAR-013) antibodies are applicable for western blot, immunohistochemical, immunocytochemical and indirect flow cytometry applications. The two former antibodies have recently been used for studying the subcellular role and distribution of Angiotensin receptors[19]. ATTO dye-labeled antibodies are also available; **Anti-Angiotensin II Receptor Type-1 (extracellular)-ATTO-550** (#AAR-011-AO) and **Anti-Angiotensin II Receptor Type-2 (extracellular)-ATTO-488** (#AAR-012-AG) antibodies are ideal for immunocytochemical and immunohistochemical applications in experiments requiring simultaneous labeling of both targets. In sddition, Alomone Labs has recently made available **Angiotensin II** (#GPA-100) to be used as a research tool.

References

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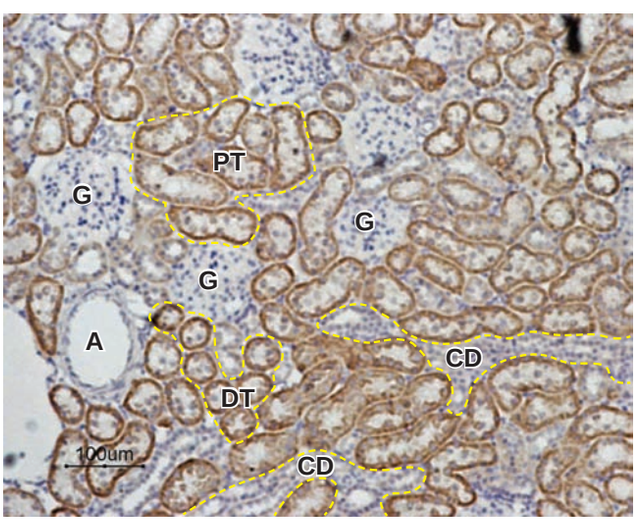
Expression of Angiotensin-(1-7) Mas Receptor in Rat Kidney.



Immunohistochemical staining of rat kidney paraffin embedded sections using **Anti-Angiotensin-(1-7) Mas Receptor** antibody (#AAR-013), (1:100). Angiotensin-(1-7) Mas Receptor (brown staining) is detected in proximal tubules (PT) and distal tubules (DT) in the renal cortex. Collecting ducts (CD) are less stained and both *glomeruli* (G) and blood vessels (A) are negative. Hematoxilin is used as the counterstain. Experimental procedure and figure processed at Alomone Labs.

Related Products

Compound	Cat. #
Angiotensin II Receptor Antibodies	
Anti-Angiotensin II Receptor Type-1 (ext.)	AAR-011
Anti-Angiotensin II Receptor Type-1 (ext.)-ATTO-550	AAR-011-AO
Anti-Angiotensin II Receptor Type-2 (ext.)	AAR-012
Anti-Angiotensin II Receptor Type-2 (ext.)-ATTO-488	AAR-012-AG
Angiotensin II Receptor Ligands	
Angiotensin II	GPA-100
Angiotensin-(1-7) Mas Receptor Antibody	
Anti-Angiotensin-(1-7) Mas Receptor	AAR-013