Neuronal Angiotensin

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Overview of Systemic Angiotensin Production

Our understanding of the renin–angiotensin system began in the late 1800s with the discovery of a pressor agent, called renin, derived from the kidney. Further work throughout the 1900s defined a humoral system in which the active agent, angiotensin (Ang II), was derived from a protein precursor, angiotensinogen (Aogen), via the sequential action of two enzymes, renin and angiotensin converting enzyme (ACE) ([Figure 1](#)). In this humoral system, Aogen is produced by the liver and secreted into the circulation. Renin, an aspartyl protease, is secreted into the plasma from cells of the juxtaglomerular apparatus in the kidney. Renin's only known substrate is Aogen, and the activity of this enzymatic reaction is considered to be the rate-limiting step in the pathway. Cleavage of Aogen yields a large glycoprotein of no known function and an inactive decapeptide, Ang I. Cleavage of Ang I by the zinc-dependent carboxypeptidase, ACE, produces the active octapeptide, Ang II. ACE is located on endothelial cells throughout the vascular system, with a particularly high concentration in the lung. Subsequent N-terminal cleavage of Ang II produces the heptapeptide Ang III, which is equipotent at Ang II receptors and may be an important ligand in some tissues.

Receptors for Ang II were cloned in the 1990s and shown to be G-protein-coupled receptors with seven transmembrane spanning domains. Two receptors, with approximately 30% homology, were cloned and termed AT_1 and AT_2 receptors. The AT_1 receptor, which mediates most of the classical actions of Ang II, was shown to occur in two isoforms in rodents, AT_{1A} and AT_{1B}. Although the number of intracellular signaling pathways employed by the AT_1 receptor continue to expand, many of the principal actions involve coupling to the $\alpha_{q/11}$ G-protein subunit. Activation results in cleavage of phosphatidyl-inositol (4,5) bisphosphate, by phospholipase C_β, to yield diacylglycerol and inositol trisphosphate with subsequent increases in intracellular calcium concentrations and activation of protein kinase C. The angiotensin AT_2 receptor signals via activation of the protein phosphatases PP2A, SH2 domain-containing phosphatase, and mitogen-activated protein kinase phosphatase 1, resulting in the dephosphorylation and inactivation of kinases including growth factor tyrosine kinase receptors and extracellular signal-regulated kinase 1 (ERK-1) and 2 (ERK-2). Antiproliferative and proapoptotic actions mediated by the AT_2 receptor appear to involve the inactivation of ERK-1 and -2 by dephosphorylation. The binding of Ang II to the AT_2 receptor has also been shown to activate nitric oxide and phospholipase A2 in certain tissues. Investigation into the signaling pathways associated with AT_2 receptor activation is further complicated by the complex physiological roles of the receptor, often in pathological states.

Studies have indicated the involvement of other enzymes and receptors in the activity of the renin–angiotensin system ([Figure 1](#)). Angiotensin converting enzyme 2 (ACE2) was cloned and found to exhibit 40% homology with ACE but with one catalytic domain. In contrast to ACE, ACE2 cleaves single amino acid residues from the C-terminus of peptides. ACE2 is thought to have a counterregulatory role in the renin–angiotensin system, being involved in the degradation of Ang II and the generation of Ang 1–7, an effector peptide that often shows opposing actions to Ang II. The receptor for Ang 1–7 was found to be the 'orphan' G-protein-coupled receptor that is encoded by the proto-oncogene *mas*. Mice with a deletion of the *mas* proto-oncogene are not responsive to Ang 1–7 and lose the binding site for Ang 1–7 in the kidney.

ACE2 is also the receptor for some coronaviruses, including the virus that causes severe acute respiratory syndrome (SARS-CoV) and another virus associated with respiratory tract infections, HCoV-NL63. ACE2 facilitates viral entry into and infection of target cells by binding specifically and with high affinity to the S protein of the viruses.

Angiotensin IV is generated from Ang III by the cleavage of the N-terminal arginine by aminopeptidase N. Angiotensin IV was initially thought to be biologically inactive because it exhibited very low affinity for the AT_1 and AT_2 receptors. However, injection of Ang IV into the cerebral ventricles enhances performance in memory tasks, and it was shown to bind to a specific, high-affinity site termed the angiotensin AT_4 receptor. Subsequent work identified the AT_4 receptor as an enzyme, insulin-regulated amino peptidase.

Tissue Renin–Angiotensin Systems

In the early 1970s, evidence of local formation of angiotensin peptides in specific tissues began to
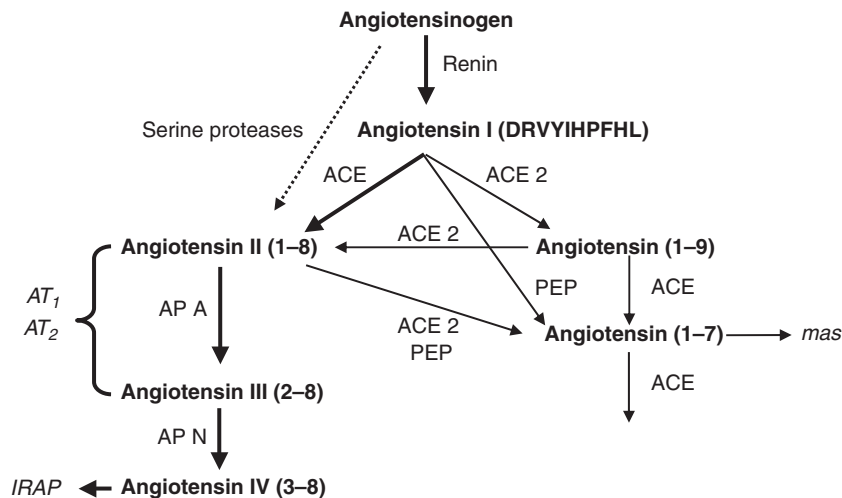
Figure 1 Overview of the enzymes and receptors involved in the production and function of angiotensin peptides. The bold arrows indicate the pathway traditionally ascribed to this system. Proteins and peptides are indicated in bold type. Enzymes are shown in normal type, and receptors are shown in italics. ACE, angiotensin converting enzyme; AP, aminopeptidase; IRAP, insulin-regulated aminopeptidase; PEP, prolyl endopeptidase.

emerge. It has since been demonstrated that many tissues, including kidney, heart, blood vessels, adrenal gland, uterus, testes, and brain, have the potential to produce Ang independently of the circulating renin–angiotensin system. Although the exact mechanism of formation remains controversial for many of these tissues, the existence of independently regulated tissue renin–angiotensin systems is widely accepted. As discussed in the following sections, there is also considerable evidence for the local generation of Ang peptides in the brain which act on receptors within the blood–brain barrier to regulate a variety of functions.

Angiotensinogen in Brain

Angiotensinogen is expressed in relatively high levels by the brain, with mRNA levels reported to be approximately one-third those produced by the liver. Measurements of Aogen concentrations in cerebrospinal fluid by radioimmunoassay indicate that it represents 2% or 3% of the total protein content. This Aogen is identical in amino acid structure to that produced by liver but may be differentially glycosylated. Most Aogen mRNA expression in the brain occurs in ependymal and astroglial cells. Interestingly, there is regional heterogeneity of expression, with highest levels in hypothalamic, midbrain, and medullary regions that are associated with the known actions of Ang peptides. Transgenic mouse lines with reporter protein expression under the control of the Aogen promoter demonstrate neuronal expression of Aogen but only in a very small number of cell groups. Most evidence indicates that Aogen synthesized by glia is constitutively secreted into the extracellular space;

however, studies have demonstrated that some Aogen might be directed to the nucleus. The relative proportion of intracellular versus secreted Aogen existing *in vivo* and the role of the intracellular protein have not been examined.

Transgenic rat and mouse models demonstrate the functional importance of brain-derived Aogen. Transgenic rats which produce an Aogen antisense under the regulation of a glial-specific promoter show multiple functional deficits, including decreased blood pressure and impaired fluid and electrolyte balance. Evidence for the converse is available in mice, in which global overexpression of renin and Aogen leads to increased blood pressure that can be reduced by glial-specific ablation of Aogen production. Together, these observations indicate that brain Aogen plays an important role in the regulation of blood pressure, but they do not shed light on how the Aogen is processed to yield Ang II and other active components of the system.

The presence of high concentrations of Aogen in astrocytes may also be suggestive of wider roles for the brain renin–angiotensin system. Investigation of functional alterations in the Aogen knockout mouse revealed impairment of blood–brain barrier function which can be restored by exogenous administration of Ang II or Ang IV. This blood–brain barrier dysfunction is thought to be due to the decreased expression of glial fibrillary acidic protein and laminin in the astrocytes of these animals.

Renin in the Brain

Efforts to demonstrate the existence and localization of renin in brain have proven extremely difficult.

Initial biochemical studies, which made every effort to remove contamination from systemic renin, demonstrated renin-like enzymatic activity in extracts of brain but did not establish the identity of renin. Several other enzymes, including cathepsin D and G, tonin, and kallikrein, have the capacity to form Ang peptides from renin substrate, and it remains a distinct possibility that enzymes other than renin might contribute to the generation of Ang peptides in brain. The use of renin antibodies in immunohistochemical and Western blot analysis to identify brain renin was also fraught with issues pertaining to antibody specificity. Initial attempts to demonstrate the existence of renin mRNA, using *in situ* hybridization or Northern blot analysis, also produced variable results, most likely due to the use of whole brain, which thus diluted any renin present in discrete brain regions to below the level of detectability. Using Northern blot hybridization methods, Dzau and colleagues found evidence for the existence of renin mRNA in brain with levels approximately 10% of those found in kidney.

In the kidney, renin is produced as preprorenin that is sequentially cleaved to form active renin. The renin gene has nine exons, the first of which encodes a signal peptide which targets the nascent protein to the endoplasmic reticulum for glycosylation and subsequent secretion from the cell. Exon 1 has three different transcriptional initiation points, which form different renin isoforms. Experiments have demonstrated two distinct renin isoforms formed by tissue-specific transcriptional initiation in brain. Initiation from exon 1a forms the classic 'renin a' found in the kidneys and systemic circulation, whereas the exon 1b transcription start point forms 'renin b.' This latter isoform lacks the coding for the signal peptide and is likely to be a constitutively active intracellular enzyme. These data raise the possibility of two distinct mechanisms of Ang peptide formation in brain – extracellular and intracellular.

However, these studies did not provide information on the distribution of renin or evidence of the cell type responsible for production. Other efforts have focused on the use of long lengths of renin promoter to drive expression of marker proteins in transgenic mice. Using a 4.1 kbp of the 5′ flanking sequence of the renin gene to drive expression of green fluorescent protein, Gross and colleagues demonstrated tissue-specific expression and regulation of the marker protein. Using this mouse model, Sigmund and colleagues localized renin expression in different regions of the mouse central nervous system. This expression occurs in many brain regions known to be involved in the regulation of fluid and electrolyte balance and autonomic control by Ang II and is predominantly localized to neurons.

Thus, current evidence supports the view that renin is expressed in the brain predominantly by neurons. The presence of renin isoforms indicates the potential for both intracellular and extracellular enzymatic activity.

Questions remain regarding the localization of renin in relation to the precursor, Aogen, and how these interact to produce Ang peptides in brain. The highest levels of renin expression occur in the cerebral and cerebellar cortices in regions where Aogen expression is low or absent. Observations in double transgenic mice, in which long sequences of the renin and Aogen promoters are used to drive expression of different reporter proteins, indicate that coexpression of renin and Aogen is rare; this is in line with observations that they are expressed in different cell types. However, adjacent cellular expression was observed in several nuclei, providing evidence to support the view that secreted Aogen might be cleaved locally in the extracellular space or taken into neighboring neurons for further processing to Ang I.

Angiotensin Converting Enzyme

Within the circulation, Ang I is rapidly converted to Ang II by the action of ACE, a zinc-dependent metallopeptidase that cleaves a dipeptide from the C-terminus of Ang I to form the effector peptide Ang II. Specific inhibitors of ACE are effective in inhibiting the formation of Ang II *in vivo* and attenuating the physiological effects of the peptide. Hence, these inhibitors have been developed very successfully into therapeutic drugs that are widely used to treat hypertension, heart failure, vascular disease, and renal disease. In addition to enzymatic cleavage of Ang I, ACE cleaves a range of other small peptide substrates, many of which are present in brain, including bradykinin, substance P, met-enkephalin, beta-endorphin, dynorphin 1–8, neurotensin, and luteinizing hormone releasing hormone.

Two isoforms of ACE exist in mammals and both are membrane bound, although a secreted form of ACE that is catalytically active is found in the circulation. The more abundant 140 kDa somatic enzyme occurs on the plasma membrane of endothelial and epithelial cells and has a large extracellular domain that contains two homologous regions, each with an active site. The testicular isoenzyme contains only one active site, which shares sequence homology with the C-terminal active site of the somatic enzyme and is the site that has been shown to selectively cleave Ang I.

In the many brain regions, ACE occurs in high concentrations. Many of these sites are also enriched with Ang II-containing cell bodies and fibers and Ang AT_1 receptors, including the circumventricular

organs, several hypothalamic nuclei, and the dorsal vagal complex in the medulla. It is proposed that at these sites, ACE participates in the local production of Ang II, either from circulating Ang I (as is the case for the circumventricular organs) or from brain-derived Ang I. However, ACE is much more abundantly distributed and high concentrations of the enzyme are detected in the basal ganglia, choroid plexus, hippocampus, cerebral cortex, and cerebellum, where its roles remain to be elucidated and may involve cleavage of other neuropeptide substrates. Although the predominant form of ACE in the brain appears to be membrane bound, a low level of the enzyme has been detected in cerebrospinal fluid, at a concentration 100-fold lower than that detected in serum.

Ang II/III Peptide Localization

Because they are cleavage products of a precursor that is secreted into the extracellular space, the cellular localization of Ang peptides in the brain has proven difficult. Immunohistochemistry has been problematic because of uncertain antibody specificity. Detailed maps of the distribution of Ang II-like immunoreactive cells and fibers have been produced but have mostly relied on results obtained with one antibody. Due to this scarcity of reliable antibodies, our knowledge of the neuronal circuitry that may utilize Ang II as a neurotransmitter is limited. However, in support of the veracity of the maps of Ang II distribution, there is a very high concordance in the distributions of Ang II-like immunoreactive nerve terminals and Ang II AT_1 receptors throughout the brain. There are some notable exceptions; for example, the magnocellular nuclei of the hypothalamus and central nucleus of the amygdala contain high levels of Ang II-like immunoreactive nerve terminals but low to undetectable concentrations of receptor.

Angiotensin AT_1 Receptors

As outlined previously, several controversies exist in relation to the production of Ang II in the brain. Some of the most convincing evidence for the existence of a brain renin–angiotensin system is derived from examination of the distribution of the Ang receptors and the physiological actions exerted through them. Unequivocal evidence points to the existence of Ang AT_1 and AT_2 receptor mRNA and binding sites (**Figure 2**) present in a characteristic distribution throughout the brain. These receptors show identical binding profiles to the peripheral receptor, including antagonism by the nonpeptide AT_1 and AT_2 receptor antagonists.

In some cases, brain AT_1 and AT_2 receptors are located outside the blood–brain barrier, where they are accessible to systemic Ang peptides. However, the vast majority of sites are behind the blood–brain barrier, where they are presumably accessed by brain-derived Ang peptides. Administration of Ang peptides into these regions generally results in neuronal excitation and a characteristic range of site-dependent physiological responses. More important, in the context of establishing a role for brain-derived Ang peptides, pharmacological blockade of these receptors with specific and selective antagonists attenuates many physiological responses, such as drinking following dehydration or autonomic activation in response to mental stress. Together, these observations provide strong support for the existence of brain-derived Ang peptides that regulate physiological functions in the central nervous system.

There is an extensive distribution of Ang II AT_1 receptors in the brain. Moderate to high levels of AT_1 receptors occur in the sensory circumventricular organs (subfornical organ and vascular organ of the lamina terminalis and area postrema); piriform cortex; lateral septum; bed nucleus of the stria terminalis; median preoptic nucleus; periventricular, parvocellular paraventricular, and dorsomedial nuclei of the hypothalamus; locus coeruleus; lateral parabrachial nucleus; nucleus of the solitary tract; spinal nucleus of the trigeminal tract; and ventrolateral medulla. Most of these sites also possess high levels of Aogen-expressing astrocytes and ACE.

Actions via AT_1 Receptors

Electrophysiological studies have demonstrated that neurons in many of the sites listed previously are responsive to local application of Ang II. The predominant response is excitation via an AT_1 receptor-mediated inhibition of outward potassium channels. Microinjection studies with exogenous Ang II and/or AT_1 receptor antagonists have identified several actions of Ang II in the brain. In brief, these include the following:

- Alterations in autonomic activity to the cardiovascular system, leading to increases in blood pressure and heart rate, through direct stimulation of efferent pathways and regulation of sensory afferent (e.g., the baroreceptor reflex) information
- Increases in fluid and salt intake
- Stimulation of the secretion of several neuroendocrine hormones, including vasopressin, adrenocortictropic hormone, and luteinizing hormone releasing hormone
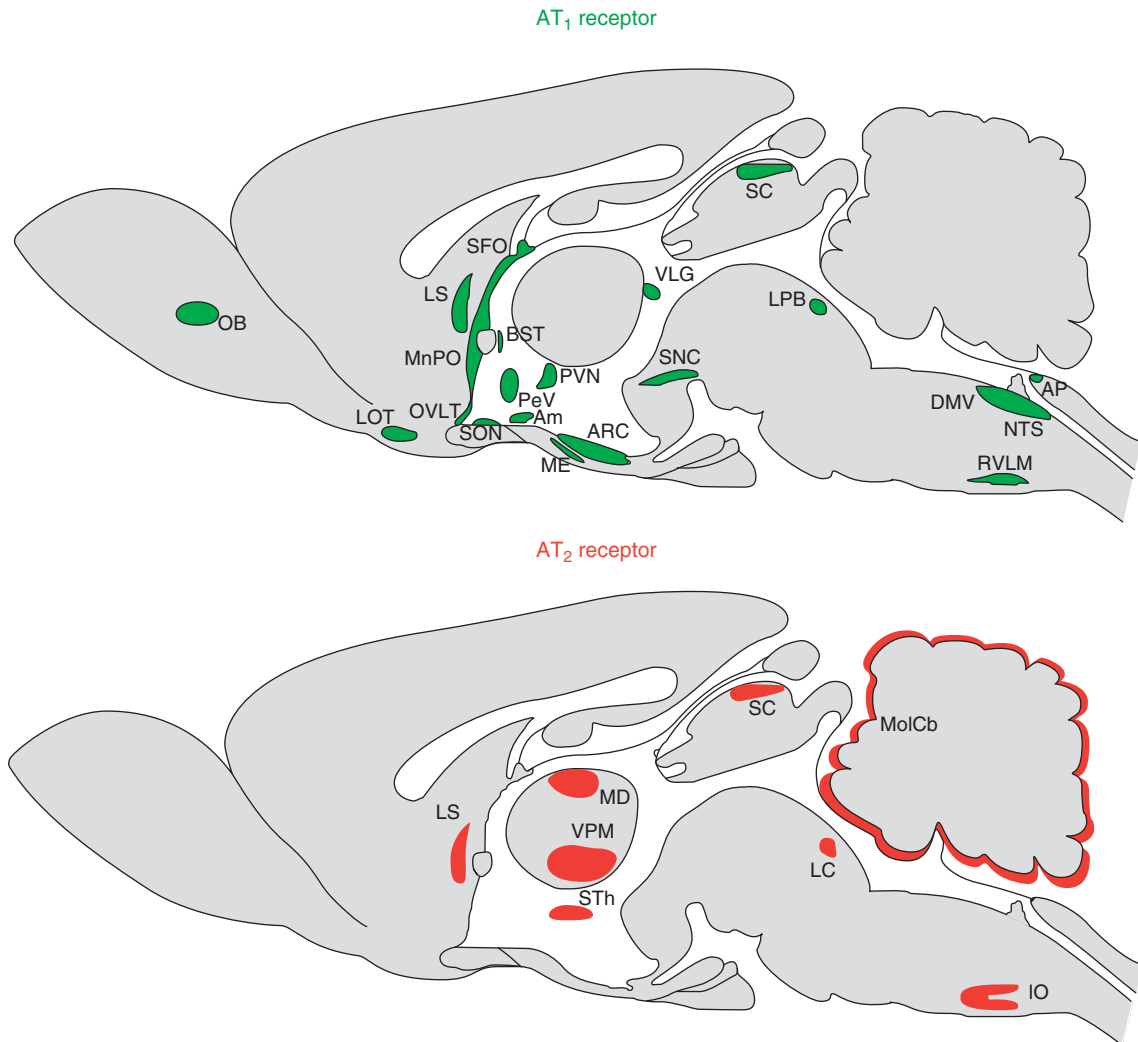- Effects on learning and memory

Figure 2 Schematic diagram representing the distribution of angiotensin AT_1 and AT_2 receptors in the brain. The distribution of receptors, determined by either *in vitro* autoradiography or *in situ* hybridization histochemistry, is depicted on midsagittal sections of the rat brain. Am, amygdala; AP, area postrema; ARC, arcuate nucleus; BST, bed nucleus of the stria terminalis; DMV, dorsal motor nucleus of the vagus; IO, inferior olivary nucleus; LC, locus coeruleus; LOT, nucleus of the lateral olfactory tract; LPB, lateral parabrachial nucleus; LS, lateral septum; MD, mediodorsal nucleus of the thalamus; ME, median eminence; MnPO, median preoptic nucleus; MolCb, molecular layer of the cerebellum; NTS, nucleus of the solitary tract; OB, olfactory bulb; OVLT, organum vasculosum of the lamina terminalis; PeV, periventricular nucleus of the hypothalamus; PVN, paraventricular nucleus of the hypothalamus; RVLM, rostral ventrolateral medulla; SC, superior colliculus; SFO, subfornical organ; SON, supraoptic nucleus; SNC, substantia nigra pars compacta; STh, subthalamic nucleus; VLG, ventrolateral geniculate nucleus; VPM, ventral posteromedial nucleus of the thalamus.

Angiotensin AT_2 Receptors in Brain

Angiotensin AT_2 receptors occur in high levels with a wide distribution in the brain during development. In the adult, this distribution becomes restricted to a few sites, including some thalamic nuclei, the cerebellum, and the inferior olivary nucleus. This distribution indicates a role in sensory information processing. The phenotype of the AT_2 receptor knockout mouse also points to some effects of Ang II on these receptors in the brain. These mice show reduced spontaneous movement and exploratory behavior, and there is evidence for effects of Ang II on cognitive function and memory. However, these are often contradictory in regard to potentiation or inhibition of memory formation. It has been proposed that the detrimental effects of Ang II on memory consolidation and retrieval are mediated by the AT_2 receptor. Further support for the angiotensin AT_2 receptor altering cognitive function comes from the detection of mutations in the coding region of this receptor in patients with mental retardation.

Ang 1–7 and Its Binding Site, the *mas* Oncogene

Angiotensin 1–7 may play a role in the central regulation of blood pressure because microinjection of Ang 1–7 into various sites alters arterial pressure and stimulates release of vasopressin. Ang 1–7 has also been reported to enhance long-term potentiation in the lateral nucleus of the amygdala and in the CA1 region of the hippocampus, suggesting a role in memory formation.

Ang IV and Its Binding Site, IRAP

Intracerebroventricular injection of Ang IV in rats enhances performance in several different memory tasks. A binding site that recognizes Ang IV with high affinity, but not Ang I, Ang II, AT_1, or AT_2 antagonists, was named the AT_4 receptor based on its distinct pharmacology. A surprising finding was the discovery that this receptor is the previously known insulin-regulated aminopeptidase (IRAP), a transmembrane zinc metallopeptidase known to traffic with the GLUT-4 glucose transporter in response to insulin. Ang IV, LVV-hemorphin-7, and other AT_4 ligands were all found to be competitive inhibitors of IRAP but were not cleaved to any appreciable extent by the enzyme. The mechanism by which Ang IV and other AT_4 ligands mediate their memory effects is yet to be resolved.

Conclusion

During the past several decades, it has been established that Ang peptides are generated in the brain. However, despite considerable effort, many questions regarding the biochemical pathways involved in the generation of this peptide system remain incompletely resolved. This system appears to have unique properties with, for example, precursor and processing enzymes existing in different cellular compartments and the potential for neurotransmitter, paracrine, and even intracrine effects. Yet despite these deficiencies in our knowledge, it is clear that Ang peptides play important physiological and pathological roles in the central nervous system regulation of a wide variety of functions, including those that complement its peripheral actions (e.g., autonomic regulation and fluid and electrolyte balance) and other more disparate functions, such as modulation of memory and blood–brain barrier formation.

See also: Angiotensin II; Angiotensin Actions on and Within Brain.

Further Reading

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