Ang II and Ang IV: Unraveling the Mechanism of Action on Synaptic Plasticity, Memory, and Epilepsy

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The central angiotensin system plays a crucial role in cardiovascular regulation. More recently, angiotensin peptides have been implicated in stress, anxiety, depression, cognition, and epilepsy. Angiotensin II (Ang II) exerts its actions through AT₁ and AT₂ receptors, while most actions of its metabolite Ang IV were believed to be independent of AT₁ or AT₂ receptor activation. A specific binding site with high affinity for Ang IV was discovered and denominated "AT₄ receptor". The beneficiary effects of AT₄ ligands in animal models for cognitive impairment and epileptic seizures initiated the search for their mechanism of action. This proved to be a challenging task, and after 20 years of research, the nature of the "AT₄ receptor" remains controversial. Insulin-regulated aminopeptidase (IRAP) was first identified as the high-affinity binding site for AT₄ ligands. Recently, the hepatocyte growth factor receptor c-MET was also proposed as a receptor for AT₄ ligands. The present review focuses on the effects of Ang II and Ang IV on synaptic transmission and plasticity, learning, memory, and epileptic seizure activity. Possible interactions of Ang IV with the classical AT₁ and AT₂ receptor subtypes are evaluated, and other potential mechanisms by which AT₄ ligands may exert their effects are discussed. Identification of these mechanisms may provide a valuable target in the development in novel drugs for the treatment of cognitive disorders and epilepsy.

Introduction

In the central nervous system (CNS), angiotensin peptides are well known to exert dipsogenic and pressor effects. More recently, the central angiotensin system has been implicated in stress, anxiety, depression, epilepsy, and cognition, as reviewed by Wright et al. [1]. Angiotensin peptides are generated from the precursor protein angiotensinogen through a cascade of enzymatic conversions. Angiotensin I (Ang I) is considered biologically inactive but its metabolites, Ang II and Ang III, are potent mediators of the dipsogenic and pressor effects of the central angiotensin system, acting as agonists of the AT₁ and AT₂ receptor subtypes [2]. Ang IV also exerts central pressor effects, albeit less potently than Ang II or Ang III [3,4]. However, Ang IV is considered of interest for therapeutic drug development since the discovery of its beneficial effects in animal models of cognitive impairment [5–11] and epileptic seizures [12–14].

A specific binding site, distinct from the classical AT₁ and AT₂ receptor subtypes, that displayed high affinity for Ang IV, was described and denominated the "AT₄ receptor" [2,15,16]. The exact nature of this binding site remains controversial. Since Ang IV mediates central pressor effects as an AT₁ receptor agonist [4,17,18], the involvement of classical angiotensin receptors in other actions of Ang IV cannot be ruled out. In 2001, Albiston et al. discovered that insulin-regulated aminopeptidase (IRAP) is a high-affinity binding site for Ang IV [19]. This breakthrough initiated the search for selective IRAP ligands and investigation of their biological effects. In 2008, Wright et al. proposed that not IRAP but the tyrosine kinase receptor c-MET is the binding site mediating the biological effects of Ang IV [1]. It is possible that distinct binding sites may independently mediate the various effects of Ang IV. Given that some of the effects of Ang II may result from its conversion to Ang IV, we will focus on the effects of both Ang II and Ang IV on synaptic

Figure 1 Overview of the major enzymatic conversions involved in the formation and degradation of Ang II and Ang IV. ACE, angiotensin converting enzyme; APA, aminopeptidase A; APN, aminopeptidase N; DAP, aspartyl aminopeptidase; APB, aminopeptidase B; Δ, cleaving site. Adapted from Refs. [19,20].

transmission and plasticity, learning, memory, seizures, and epilepsy. Delineation of the binding sites responsible for these effects may facilitate assessment of their potential as targets for the development of novel treatments for memory impairment and epilepsy.

Formation of Ang II and Ang IV in the CNS

Enzymes Involved in the Formation of Ang II and Ang IV

The enzymatic cascades involved in the formation and degradation of angiotensin peptides in the brain are elaborated in recent reviews by Karamyan and Speth [20,21]. The present review focuses on a major enzymatic pathway involved in the central formation of Ang II and Ang IV (Fig. 1). The angiotensinogen precursor protein is typically metabolized by renin into the decapeptide Ang I. However, in the brain, other enzymes such as tonin were identified as potential Ang I-generating enzymes [20,21]. Angiotensin-converting enzyme (ACE) cleaves the carboxyterminal His-Leu residues from Ang I, converting it to Ang II [22]. Alternatively, tonin can directly convert angiotensinogen to Ang II [21,23,24]. Aminopeptidase A (APA) is proposed as a major Ang II-metabolizing enzyme, removing the aminoterminal Asp residue to form Ang III [25,26]. In turn, aminopeptidase N (APN) is suggested as the dominant Ang III-metabolizing enzyme, removing its aminoterminal Arg residue to form Ang IV

[25,27]. However, other enzymes may contribute to the conversion of Ang II and Ang III in the brain such as aspartyl aminopeptidase (DAP) and aminopeptidase B (APB) [20,21,28,29]. Ang IV can then be further degraded into aminoterminal-deleted peptides by enzymes such as APN and DAP [30].

Ang II, Ang III, and Ang IV have been characterized as bioactive angiotensin fragments. Ang II and Ang III exert their effects via the AT₁ and AT₂ receptor subtypes. Ang III was proposed to be the major regulator of central pressor effects instead of Ang II, based on the observation that the APA inhibitor EC33 exerts a central blood pressure-lowering effect, whereas the APN inhibitors EC27 and PC18 have central pressor effects [25]. However, this hypothesis is actively debated. It was recently demonstrated that degradation-resistant Ang II analogs exert a pressor response similar to Ang II without being converted to Ang III [31]. Furthermore, hypertension and hypersensitivity to Ang II-mediated pressor effects were previously reported in APA knockout mice [32]. Ang IV has a less prominent role in cardiovascular regulation but mediates specific biological effects independently of AT₁ and AT₂ receptors through its putative AT₄ receptor.

Neuronal and Glial Localization of the Central Angiotensin System

The aforementioned components involved in the generation of angiotensin peptides are all expressed within the

CNS. However, their precise cellular localization has been a matter of debate [33]. It appears that both neurons and glia can produce angiotensin peptides but may differentially contribute to the function of these peptides [34].

Localization of Angiotensinogen and Its Metabolizing Enzymes

Angiotensinogen mRNA was exclusively found in astroglia, while protein immunoreactivity was localized to both astroglia and, to a lesser degree, to neurons [35]. Electron microscopy revealed angiotensinogen staining in astroglia and adjacent neuronal dendrites, suggesting that astroglia are the production site for this protein, and that neurons are capable of angiotensinogen uptake [36]. Reduction of arterial pressure after glial-specific ablation of angiotensinogen in transgenic mice further supports a functional role for angiotensinogen production in glial cells [37]. In contrast, renin mRNA and immunoreactivity were localized to neurons throughout the brain [38–41]. Transgenic mice expressing enhanced green fluorescent protein under the control of the mouse renin promoter confirmed that neurons are the principal site of renin production [42]. Secreted or nonsecreted isoforms of renin provide a model for extracellular and neuronal generation of angiotensin peptides [43]. However, tonin may be a glial alternative to renin as it is capable of directly converting angiotensinogen to Ang II, thus providing a basis for glial formation of angiotensin fragments [24]. ACE is present in epithelial cells of the choroid plexus, the ependymal cells of the cerebral ventricles, and the endothelial surface of blood vessels [44]. Using electron microscopy, ACE was also detected in astroglial processes surrounding dendrites and in neuronal soma, dendrites, and synaptic boutons [36,45]. Within synaptic boutons, ACE staining was limited to presynaptic and postsynaptic membranes [45].

Localization of Ang II

Ang II has been demonstrated in both astroglia and neurons [46,47]. Ang II immunoreactive nerve fibers and terminals are widely distributed throughout the brain [33]. Ang II immunoreactive pinocytotic vesicles in dendrites suggest that neurons are capable of Ang II uptake from the extracellular space [46,47]. Neuronal synthesis of Ang II has been demonstrated in transgenic mice expressing human angiotensinogen under its own promoter and human renin under a neuron-specific promoter [43]. Ang II immunoreactivity is present at the plasma membranes of dendrites and axon terminals without synaptic specialization, but is also found in large, dense, core vesicles at axodendritic synapses [46,47]. This suggests that Ang II is a neuropeptide that may activate its local recep-

tors on axosynaptic membranes through synaptic transmission and distant receptors through volume transmission. However, Ang II release upon neuronal burst firing is yet to be demonstrated. In addition to release from axon terminals, release from neuronal soma and dendrites, production by astroglia, and extracellular synthesis can be proposed.

Localization of Ang IV

The distribution of Ang IV throughout the brain has not yet been investigated. APA and APN are primarily located to the adventitial surface of microvessels throughout the brain [48–50]. Since neurons, glial cells, and endothelial cells were not labeled, selective expression on pericytes was suggested for APA and confirmed for APN [48–50]. The localization of both aminopeptidases to the plasma membrane of pericytes suggests that Ang IV formation is predominantly localized to the extracellular space surrounding the microvessels in the brain. These microvessels may thus be potential targets for endogenous Ang IV. Correspondingly, an increase in cerebral microcirculation was observed after local administration of Ang IV [51–53]. Since APN is proposed as a predominant enzyme involved in both the formation and the degradation of Ang IV, it can be proposed that once formed, Ang IV is rapidly degraded to smaller peptides. However, intracellular formation of Ang IV may be mediated through other enzymes such as APB and DAP. Using the microdialysis technique and a highly sensitive liquid chromatography-mass spectrometry system, we were only able to measure Ang IV immediately after probe insertion [54,55]. After restoration of the tissue integrity, extracellular levels of Ang IV dropped below the detection limit of 50 pM [54,55]. This suggests the intracellular presence of Ang IV. It is uncertain whether Ang IV acts as a classical neuropeptide, but a myriad of intriguing effects on the nervous system have been observed after exogenous administration of Ang IV, prompting the investigation to determine its specific binding site.

The AT₄ Receptor and Its Ligands

The presence of a binding site with nanomolar affinity for Ang IV was first reported in 1992 by Swanson *et al.* in membranes prepared from bovine adrenal cortex [15] and Harding *et al.* in the guinea pig hippocampus [16]. The pharmacological profile of this binding site was clearly distinct from the classical angiotensin AT₁ and AT₂ receptors since the binding of ¹²⁵I-Ang IV was not displaced by either the nonselective peptidic angiotensin antagonists [Sar¹,Ile⁸]-Ang II and [Sar¹,Ala⁸]-Ang II, or the selective non-peptidic AT₁ receptor antagonist losartan, the AT₂ receptor antagonist PD123177, or the AT₂ re-

Table 1 Structure of AT₄ ligands and putative receptor activity Adapted from Ref. [7]

Ligand	Structure	Putative activity
Ang IV	Val-Tyr-Ile-His-Pro-Phe	Agonist
Nle ¹ -Ang IV	Nle-Tyr-Ile-His-Pro-Phe	Agonist
Norleucinal ¹ -Ang IV	Nle ψ Tyr-Ile-His-Pro-Phe	Agonist
Nle ¹ ,Leu ³ -Ang IV	Nle-Tyr-Leu ψ His-Pro-Phe	Antagonist
Divalinal-Ang IV	Val ψ Tyr-Val ψ His-Pro-Phe	Antagonist
LVV-H7	Leu-Val-Val-Tyr-Pro-Trp-Thr-Gln-Arg-Tyr	Agonist

ψ , CH₂-NH bond.

ceptor agonist CGP42112A [15,16,56]. This binding site displayed a broad tissue distribution and was found in different mammalian species. Incubation of guinea pig brain slices with ¹²⁵I-Ang IV revealed that the AT₄ receptor subtype is widely distributed in the brain, with a high density not only in areas involved in cognition and epilepsy such as the hippocampus, the basolateral amygdala, the cerebral cortex, and the septum but also in motor neurons and motor nuclei [57]. A similar distribution of ¹²⁵I-Ang IV binding sites was shown in mice, rat, macaque, and human brain slices [58–61].

The discovery of this binding site in combination with the biological effects of Ang IV led to the concept of the AT₄ receptor [2,15,16]. These pioneering binding studies also paved the way to define the structural requirements for angiotensin-derived peptides to compete with ¹²⁵I-Ang IV binding [62]. Three N-terminal residues of Ang IV were found critical for ligand binding. An N-terminal primary α -amine and an L-conformation for the first amino acid α -carbon atom [63], an activated aromatic ring in the side chain of the second amino acid, and a hydrophobic amino acid in the third position are required for high-affinity binding [64]. Hydrophobic substitution of the first amino acid produced the greatest binding affinity for the AT₄ receptor. As such, modification of the amino acid, valine, to a straight-chain aliphatic moiety with four carbon atoms produced the putative agonist norleucine¹-Ang IV (Nle¹-Ang IV), which has a higher affinity for the AT₄ receptor compared to native Ang IV (Table 1). Conversion of the first CO-NH peptide bond of Nle¹-Ang IV with a CH₂-NH reduced peptide bond produced Nle¹-Ang IV, a nanomolar affinity agonist with potential resistance to enzymatic degradation. Similarly, the putative antagonist divalinal-Ang IV was formed through substitution of the third amino acid, isoleucine, by valine and reduction of the first and third amide bonds of Ang IV. The putative antagonist Nle¹,Leu³-Ang IV was synthesized by substitution of the third amino acid, isoleucine, by leucine and reduction of the third peptide bond in Nle¹-Ang IV [62,63,65,66]. Since divalinal-Ang IV does not bind to AT₁ or AT₂ receptors, has a nanomolar affinity for the

AT₄ receptor, and blocks several effects of Ang IV and Nle¹-Ang IV, it was proposed as the first selective AT₄ receptor antagonist [66].

In addition to these Ang IV-derived peptides, a structurally distinct fragment of the hemoglobin β -chain, Leu-Val-Val-hemorphin 7 (LVV-H7), was isolated from sheep brain and found to compete with ¹²⁵I-Ang IV for AT₄ receptor binding. Moreover, the binding properties and distribution of radiolabeled LVV-H7 in brain slices were identical to those of Ang IV. Since LVV-H7 mimics the central effects of Ang IV and does not bind to the AT₁ and AT₂ receptor subtypes, it was proposed as an endogenous AT₄ receptor ligand [11,67–71].

The AT₄ Receptor Controversy

Several groups attempted to identify and characterize the AT₄ receptor. In cross-linking studies using N₃-Phe⁶-Ang IV, Benzoyl-Phe⁶-Ang IV, ¹²⁵I-Benzoyl-Phe⁶-Ang IV or ¹²⁵I-Nle¹-Benzoyl-Phe⁶-Gly⁷-Ang IV as specific high-affinity photolabels, the AT₄ receptor was characterized as a 160–180 kDa membrane-bound glycoprotein, associated with a smaller 60–70 kDa subunit through disulfide linkages in bovine aorta, adrenals, kidney, heart, thymus, bladder, and human SK-N-MC neuroblastoma cells [72–75]. The bovine hippocampal AT₄ receptor appeared smaller, with a molecular weight of 150 kDa, suggesting a differentially glycosylated form that was not associated with a smaller subunit [72,73]. However, the nature of the AT₄ receptor remains controversial. Both IRAP and c-MET have been proposed as high-affinity binding sites, but conclusive evidence for the involvement of either in the physiological effects of AT₄ ligands is lacking. Furthermore, it has been demonstrated that Ang IV may exert some of its effects through activation of the AT₁ receptor.

IRAP Is a High-Affinity Binding Site for AT₄ Ligands

A major breakthrough in the identification of the AT₄ receptor was accomplished by the purification and

partial sequencing of Ang IV-binding proteins from bovine adrenal membranes and their identification as IRAP [19]. HEK 293T cells transfected with IRAP exhibited typical AT₄ receptor binding characteristics, and the distribution of the IRAP mRNA and protein in the brain, visualized by *in situ* hybridization and immunohistochemistry, matched that of AT₄ receptor binding determined by ¹²⁵I-Ang IV radioligand binding [19,76].

IRAP received different denominations, gp160, vp165, oxytocinase, cystinyl aminopeptidase, or placental leucine aminopeptidase depending on where it was independently cloned. It is a protein of 1025 amino acid residues, with a 110 amino acids N-terminal hydrophilic intracellular domain, which features two dileucine motifs with preceding acidic clusters, associated with trafficking and endocytosis. The single hydrophobic 22 amino acid α -helix transmembrane domain is followed by an 893 amino acid C-terminal extracellular domain containing the catalytic site. This catalytic site consists of a GAMES motif and the HEXXH(X)₁₈ Zn²⁺-binding motif [77–79]. IRAP is classified as a type 2 transmembrane protein of the gluzincin aminopeptidase family [80]. This family includes homologous aminopeptidases such as APA and APN. *In vitro*, IRAP was demonstrated to cleave the N-terminal amino acid from several bioactive peptides including oxytocin, Arg-vasopressin, lys-bradykinin, met-enkephalin, dynorphin A, neurokinin A, neurotensin B, somatostatin, and cholecystokinin-8 [81–84]. The physiological role of IRAP in the control of oxytocin levels has not been conclusively demonstrated, despite its denomination as oxytocinase. However, the related peptide Arg-vasopressin was recently identified as a physiological substrate of IRAP *in vivo* [85].

The potential mechanisms by which IRAP may mediate the various effects triggered by AT₄ ligands were recently reviewed by Albiston et al. [86]. One hypothesis is based on the observation that AT₄ ligands are potent competitive inhibitors of IRAP *in vitro* [84]. Therefore, AT₄ ligands may exert their effects indirectly, by blocking the enzymatic activity of IRAP and preventing the degradation of its neuropeptide substrates. This hypothesis is hard to reconcile with the aforementioned concept of agonists and antagonists properties of AT₄ ligands. Divalinal-Ang IV is indeed an IRAP inhibitor but can nevertheless block some of the effects of other AT₄ ligands [53,69,87,88]. However, the characterization of divalinal-Ang IV as an AT₄ receptor antagonist is not conclusive as it has been demonstrated to mimic the effects of other AT₄ ligands in different experimental setups [71,89]. Furthermore, we recently demonstrated that the effect of Ang IV on pilocarpine-induced seizures can be attenuated by a somatostatin SST₂ receptor antagonist, suggesting that

Ang IV may exert this effect through inhibition of IRAP and the breakdown of its substrate somatostatin [12].

In several studies, AT₄ ligands were found to elicit rapid intracellular signaling events that are typically associated with receptor function, such as transient increases in intracellular Ca²⁺ and phosphorylation of ERK1/2, p38 kinase, p125-focal adhesion kinase, and p-68 paxillin in renal cell lines [89–92] or activation of the nuclear factor kappa B in vascular smooth muscle cells [93]. It can be argued that these effects may be cell type specific. However, AT₄ ligands also induced rapid effects on Ca²⁺ influx in rat hippocampal neurons and excitability of hippocampal neurons [94–97]. Since accumulation of substrate peptides is unlikely to result in physiological effects within a short time frame, it was proposed that binding of AT₄ ligands to IRAP may lead to the activation of signaling molecules [98,99]. Homodimer formation is a characteristic property of membrane-bound metalloproteases [100]. As a dimer, IRAP could convey information across the cell membrane. Dimer formation has been ascribed to the structurally related APN, which was able to mediate an IP₃-linked increase of intracellular Ca²⁺ and phosphorylation of MAP kinases in monocytes upon the binding of monoclonal antibodies [101,102]. To date, no direct evidence has been provided for IRAP-mediated intracellular signaling.

A role in neuronal glucose uptake has also been proposed. IRAP was first identified in rat adipocytes as a marker of vesicles containing the insulin-responsive glucose transporter GLUT₄ [78]. A potential role of IRAP in GLUT₄ tethering and trafficking was suggested based on the observation that microinjection of a cytoplasmic fragment of IRAP, expressed as a glutathione-S-transferase fusion protein, into 3T3-L1 adipocytes increased the amount of GLUT₄ at the plasma membrane [103]. The carboxyterminal domain of the GLUT₄ transporter contains a dileucine motif followed by an acidic cluster, which is required for correct intracellular targeting [104,105]. Similarly, the dileucine motif of IRAP is necessary for its intracellular retention within an insulin-responsive compartment [106]. IRAP interacts with several trafficking and tethering proteins such as tankyrase, formin homolog overexpressed in spleen, acyl-coenzyme A dehydrogenase, p115, and Akt substrate of 160 kDa, as reviewed by Albiston et al. [86]. In hippocampal pyramidal neurons, IRAP [107] and the GLUT₄ glucose transporter [108,109] are localized to secretory vesicles, analogous to the insulin-responsive vesicles of fat and muscle tissue. Based on this analogy, Fernando et al. suggested that IRAP may have a role in neuronal GLUT₄ trafficking and associated glucose uptake [107]. Consequently, AT₄ ligands were proposed to prolong the presence of IRAP

and GLUT₄ at the cell surface [86] and as such facilitate glucose uptake into neurons.

c-MET Is a High-Affinity Binding Site for AT₄ Ligands

Based on the homology between Ang IV and a particular region of the hepatocyte growth factor (HGF), Wright et al. recently proposed that the HGF receptor c-Met should instead be considered as the primary target for Ang IV [1]. Consistent with the structure of the AT₄ receptor, c-MET is a tyrosine kinase receptor with a 50 kDa α -subunit and a 145 kDa β -subunit [110]. c-Met belongs to the family of type 1 tyrosine kinase receptors and is associated with a broad range of physiological actions including stem cell proliferation and differentiation, angiogenesis by vascular endothelial cells, the growth of tumor cells, and learning and memory consolidation. The putative AT₄ receptor antagonist Nle¹,Leu³-Ang IV competed with ¹²⁵I-HGF binding to c-Met with a picomolar affinity and inhibited HGF-dependent proliferation, invasiveness, and scattering in several tumour cell lines [1].

The AT₁ Receptor May Contribute to Some of the Effects of Ang IV

A number of effects observed after intracerebroventricular (i.c.v.) administration of Ang IV can be blocked by losartan or candesartan and have therefore been attributed to the activation of central AT₁ receptors. Ang II is known to stimulate drinking behavior, vasopressin release, and sympathetic outflow [111,112], with the AT₁ receptor being the main receptor subtype involved in these effects [113]. The AT₂ receptor subtype was found to act synergistically in the dipsogenic effect but antagonistically in the pressor effects of Ang II [114]. Similarly, i.c.v. administration of Ang IV can also induce a transient drinking response [115], albeit less potently than Ang II [3], and an increase in arterial blood pressure [4,17]. The pressor effect of Ang IV was reversible by losartan [17] and candesartan [4]. Additionally, it was demonstrated that transgenic mice chronically overexpressing Ang IV under the brain-specific human glial fibrillary acidic protein promoter had an increased systolic blood pressure that was blocked by candesartan [18].

Ang IV is a full agonist for the AT₁ receptor, albeit with micromolar affinity. The EC₅₀ value for IP₃ production in human AT₁ receptor-transfected CHO-K1 and HEK293 cells was approximately 1 μ M for Ang IV as compared to 1 nM for Ang II [116,117]. In these studies, a site-directed mutation in the AT₁ receptor of Asn¹¹¹ to Gly¹¹¹ caused a dramatic decrease of the EC₅₀ for

Ang IV-mediated IP₃ production to approximately 1 nM [116,117]. The mutant AT₁ receptor was proposed to exist in a preactivated conformation with equal affinity for both Ang II and Ang IV [116,117]. Presently, there is no direct evidence for physiologically preactivated AT₁ receptors. However, in AT₁ receptor-expressing CHO cells, it was demonstrated that incubation with 10 nM Ang II induced a transient AT₁ receptor-mediated increase in intracellular Ca²⁺, whereas 100 nM Ang IV had no effect. However, when these cells were incubated with 100 nM of Ang IV after preincubation with 10 nM Ang II, an AT₁ receptor-mediated increase in intracellular Ca²⁺ was observed [18].

Effects of Ang II and Ang IV on Synaptic Transmission and Plasticity

It is well known that angiotensin peptides modulate synaptic transmission [118]. Ang II was demonstrated to excite hippocampal CA1 pyramidal neurons through disinhibition. This effect was blocked by the peptidic AT₁ and AT₂ antagonist [Sar¹,Ile⁸]-Ang II [119]. The inhibitory action of Ang II on K⁺-evoked gamma-aminobutyric acid (GABA) release from hippocampal slices was consistent with this observation [120]. Similarly, Ang IV enhanced baseline synaptic transmission in the CA1 area of the hippocampus [97,121]. Iontophoretic administration of Ang II or Ang IV into the CA3 area of anaesthetized rats predominantly increased the firing frequency of hippocampal neurons. In this study, losartan blocked the effects of Ang II but not of Ang IV, whereas divalinal-Ang IV blocked the effects of Ang IV but not of Ang II [94]. This suggests an excitatory action of both Ang II and Ang IV on hippocampal neurons of the CA1 and CA3. However, Ang II but not Ang IV suppressed basal synaptic transmission in the dentate gyrus of rat hippocampal slices [122]. In contrast, Nle¹-Ang IV increased, whereas Ang II decreased the amplitude of field potentials in the lateral amygdala [88]. This demonstrates that Ang II and Ang IV or its analogs may differentially affect neuronal excitability in different brain regions.

Similarly, it can be proposed that both peptides may have different effect on synaptic plasticity, depending on the brain area in question. Long-term potentiation (LTP) and long-term depression (LTD) are defined as a persisting enhancement or suppression of synaptic efficacy [123,124]. These forms of synaptic plasticity are posited as the underlying cellular mechanism for memory formation and extinction [125] and are strikingly similar to the synaptic rearrangements observed in the kindling model for epileptogenesis [126–128].

Table 2 Effects of Ang II and Ang IV on synaptic plasticity *in vitro*

Ligand	Location	Concentration	Effect	Receptor	Mechanism	Reference
Ang II	DG	5–50 nM	LTP ↓	AT ₁	ND	[122]
	LA	10 μ M	LTP ↓	AT ₁	ND	[129]
	LA	5–10 μ M	LTD ↓	AT ₁	VDCC	[130]
Nle ¹ -Ang IV	CA1	0.1 μ M	-	NA	NA	[95]
	CA1	1 μ M	LTP ↑	AT ₄	VDCC	[95,97,121]
	CA1	10 μ M	-	NA	NA	[95]

DG, dentate gyrus; LA, lateral amygdala; CA1, cornu ammonis region 1 of the hippocampus; VDCC, voltage-dependent Ca²⁺ channel; ↑, enhancement; ↓, suppression; ND, not determined; NA, not applicable.

Effects of Ang II and Ang IV on Synaptic Plasticity *In Vitro*

A bath application of 500 nM Ang II suppressed basal synaptic transmission in the dentate gyrus in rat hippocampal slices [122]. Ang II had no effect on basal synaptic transmission when applied at a concentration of 5–50 nM but blocked LTP induction in the dentate gyrus of rat hippocampal slices [122]. Application of 5–10 μ M Ang II suppressed the induction of LTP and stabilization of LTD within the lateral nucleus of the amygdala in horizontal rat brain slices [129,130] (Table 2). The effects of Ang II on synaptic plasticity in the hippocampus and amygdala were blocked by the AT₁ receptor antagonist losartan [122,129,130]. The mechanism underlying the effect of AT₁ receptor activation on LTP expression in the dentate gyrus and lateral amygdala are yet to be determined; however, the AT₁ receptor-dependent suppressive effect of Ang II on LTD in the lateral amygdala was blocked by nifedipine, suggesting the involvement of L-type Ca²⁺ channels (VDCC) [130].

In rat hippocampal slices, Nle¹-Ang IV enhanced baseline synaptic transmission and potentiated LTP expression in the hippocampal CA1 region [95,97,121]. A bell-shaped pharmacological profile was observed with an optimal concentration of 1 μ M for Nle¹-Ang IV [95] (Table 2). Pretreatment with the putative AT₄ receptor antagonist Nle¹-Leu³-Ang IV prevented stabilization of LTP and effectively blocked both the enhancement of baseline synaptic transmission and facilitation of LTP induction evoked by Nle¹-Ang IV [95,97,121]. In the hippocampal CA1 region, induction of LTP requires an increase in the postsynaptic Ca²⁺ concentration [131]. This influx of Ca²⁺ in CA1 neurons is primarily mediated through N-methyl-D-aspartate (NMDA) receptor channels and, to a lesser extent, through VDCC [132,133]. The facilitatory effect of Nle¹-Ang IV on baseline synaptic transmission was mediated through AMPA receptors and associated with a neuronal influx of extracellular Ca²⁺ through VDCC [97]. The induction of LTP was blocked by the NMDA receptor antagonist D,L-AP5, but coad-

ministration of Nle¹-Ang IV allowed the expression of an NMDA-independent form of LTP [97]. VDCC blockers were able to inhibit the effect of Nle¹-Ang IV on LTP induction [97].

Effects of Ang II and Ang IV on Synaptic Plasticity *In Vivo*

Injection of 5 pmol Ang II directly above the dorsal hippocampus of anaesthetized rats suppressed LTP induction in the dentate gyrus, when the injection was timed 90–120 min before high-frequency stimulation of the perforant path [134–136]. This effect was dependent on the dose and timing of administration and was blocked by the AT₁ receptor antagonist losartan [135] (Table 3). These observations confirmed previous results of this group obtained in rat hippocampal slices. The mechanism underlying the effects of AT₁ receptor activation on LTP induction in the dentate gyrus remains to be elucidated.

Both Ang IV and Nle¹-Ang IV facilitated LTP induction in dentate granule cells when injected at a dose of 5 fmol, directly above the dorsal hippocampus of anaesthetized rats, 5–15 min before high-frequency stimulation of the perforant path [96]. However, when administered 90 min before LTP induction, both Ang IV and Nle¹-Ang IV suppressed dentate LTP. Both the facilitatory and inhibitory effects of Ang IV exhibited a bell-shaped dose-dependent pharmacological profile (Table 3). Pretreatment with the putative AT₄ receptor antagonist divalinal-Ang IV did not affect LTP expression, but attenuated the short-term facilitatory and long-term inhibitory effects of Ang IV and Nle¹-Ang IV on LTP [96]. Surprisingly, losartan also antagonized the effect of Ang IV on LTP, despite the low dose of Ang IV used in this study [96]. This effect of losartan remains unexplained and has not been further investigated.

Potential Mechanisms for Facilitation of Synaptic Plasticity by AT₄ Ligands

It is tempting to describe the effects of Ang II and Ang IV on synaptic plasticity as inhibitory and facilitatory,

Table 3 Effects of Ang II, Ang IV, and Nle¹-Ang IV injection into the dorsal hippocampus on LTP induction in the dentate gyrus *in vivo*

Ligand	Dose	Effect	Time (min) before LTP induction						Receptor	Reference
			5	15	30	60	90	120		
Ang II	5 pmol	LTP ↓	ND	ND	ND	-	↓	↓	AT ₁	[136]
Ang IV	2.5 fmol	-	-	ND	ND	ND	-	ND	NA	[96]
	5 fmol	LTP ↑↓	↑	↑	-	-	↓	-	AT ₄ /AT ₁ ?	[96]
	10 fmol	-	-	ND	ND	ND	-	ND	NA	[96]
Nle ¹ -Ang IV	5 fmol	LTP ↑↓	↑	↑	-	-	↓	-	AT ₄ /AT ₁ ?	[96]

↑, enhancement of LTP; ↓, suppression of LTP; -, no effect on LTP; ND, not determined; NA, not applicable.

respectively. However, this ambiguity often reflects the effects of Ang II and Ang IV in different brain areas. As discussed previously, Ang II and Ang IV indeed exert opposite effects on the excitability of the lateral amygdala through different receptor subtypes [88], but have similar effects on synaptic transmission in the CA1 and CA3 regions of the hippocampus [94,119] and synaptic plasticity in the dentate gyrus at certain time points [96,136]. These similarities are expected, given that Ang II is rapidly metabolized to Ang IV. The half-lives of Ang II and Ang III after i.c.v. administration were reported as 23 s and 8 s, respectively [137]. However, all effects of Ang II on synaptic plasticity could be blocked by losartan, and in one study, losartan also blocked the effects of Ang IV [96]. This suggests that the analogous actions may be due to interaction with the AT₁ receptor. This remains controversial as putative AT₄ antagonists, which do not interact with AT₁ receptors, were able to block the effects of Ang IV or Nle¹-Ang IV in all studies. Furthermore, Nle¹-Ang IV was found to reverse the suppressive effect of ethanol on LTP in the CA1 of rat hippocampal slices [121], whereas the suppressive effects of ethanol on dentate LTP induction was AT₁ receptor dependent in anaesthetized rats [138]. Taken together, these data suggest that a complex interaction may exist between the AT₁ and AT₄ receptors.

Several hypotheses can be proposed to explain how interactions with AT₄ receptors may modulate synaptic plasticity. Since AT₄ ligands are competitive inhibitors of IRAP, they may modulate LTP by slowing the degradation of its substrates. Arg-vasopressin was proposed as a physiological IRAP substrate [85] and facilitated LTP in the CA1 of rat hippocampal slices [139] and in the rat dentate gyrus (Dubrovsky et al., 2003) [140]. At higher concentrations, Arg-vasopressin inhibited LTP in the CA1 of guinea pig hippocampal slices [141]. Oxytocin enhanced LTP in the CA1 of female mice hippocampal slices [142] and induced LTD in the male rat dentate gyrus [143]. Cholecystinin-8 did not affect LTP induction, but prolonged LTP expression in the CA1 of rat and guinea pig

hippocampal slices [144,145]. Other potential IRAP substrates suppressed synaptic plasticity. Somatostatin suppressed LTP and Ca²⁺ signaling in the mouse dentate gyrus [146], and endogenous dynorphin was demonstrated to block LTP induction in the guinea pig dentate gyrus [147]. Since different potential IRAP substrates may have opposite effects on LTP, a hypothetical net result of IRAP inhibition would be difficult to predict, and biphasic effects may occur. However, this hypothesis cannot explain the antagonistic properties of divalinal-Ang IV and Nle¹-Leu³-Ang IV.

Alternatively, AT₄ ligands may facilitate neuronal glucose uptake through interaction with IRAP. Intrahippocampal administration of glucose was demonstrated to activate the rapamycin-sensitive kinase mammalian target of rapamycin (mTOR) [148]. Activation of the mTOR pathway has a crucial role in the translation of proteins necessary for expression of the late phase of LTP [149]. Activation of the mTOR pathway was blocked by inhibitors of voltage-dependent Ca²⁺ channels in isolated neurons [150], and inactivation of the mTOR pathway blocked late LTP in the CA1 region of hippocampal slices [151,152]. This suggests facilitation of neuronal glucose uptake, and subsequent activation of the mTOR pathway may contribute to the effects of AT₄ ligands. In support of this hypothesis, Ang IV was found to enhance phosphorylation of the E4 binding protein 1 substrate of mTOR in endothelial cells [153]. However, since mTOR activation is downstream to Ca²⁺ influx, this cannot explain the activation of L-type Ca²⁺ channels.

In isolated neurons, HGF release was induced by glutamate treatment [154], and HGF and its receptor c-MET are clustered in excitatory synapses of the hippocampus, where c-MET colocalizes with the NMDA receptor at the postsynaptic density [155]. Exogenously applied HGF enhanced NMDA receptor-mediated LTP in the CA1 region of hippocampal slices but did not affect LTD [156]. In contrast, AT₄ ligands facilitated an NMDA receptor-independent form of LTP [97].

Effects of Ang II and Ang IV on Learning and Memory

The phenomenon of LTP is widely regarded as an important molecular mechanism for learning and memory. After 30 years of intensive research, it was only recently demonstrated that learning induces LTP in the hippocampal CA1 region *in vivo* [157]. The hippocampus plays a focal role in spatial learning and memory and a more general role in the transition from short-term to long-term episodic memory, acting as an associator of temporal or spatial discontinuous events [158–160]. The amygdala is crucial in fear learning and memory and is implicated in the acquisition and consolidation of the affect association of episodic memory [161,162]. The effects of Ang II and Ang IV on synaptic plasticity may therefore form the neurophysiological basis for their modulatory actions on learning and memory.

The effects of Ang II and Ang IV on learning and memory were extensively studied not only in the fear-motivated passive and active avoidance tasks but also in the novel object recognition and spatial short-term and long-term memory tasks. The effects of Ang II are dependent on the dose, timing, and site of administration and vary between memory tasks. Furthermore, fear motivated-memory tasks are sensitive to bias due to anxiogenic or anxiolytic effects of the studied compounds. Indeed, intrahippocampal injection of Ang II produced an anxiolytic effect in the elevated plus maze at a dose of 0.1 nmol but not at higher doses [163,164]. In contrast, an anxiogenic effect was observed in the elevated plus maze after i.c.v. administration of Ang II [165–167]. A similar anxiogenic effect was observed after i.c.v. administration of Ang IV [168–171]. However, Ang IV elicited reproducible memory-enhancing effects and reversed memory deficits in several rat models for cognitive impairment, suggesting a potential therapeutic role for its corresponding binding site.

Ang II and Ang IV may exert their effects on learning and memory through different brain areas since it was demonstrated that Ang II and Ang IV differentially increased c-Fos-like expression upon i.c.v. injection [61]. Ang IV predominantly increased c-Fos immunoreactivity in the hippocampus and piriform cortex. This increase in immunoreactivity was unaffected by pretreatment with losartan or PD123177, but was blocked by divalinal-Ang IV. In contrast, Ang II increased c-Fos-like immunoreactivity in the circumventricular organs, subfornical organ, lateral hypothalamus, and amygdala. Pretreatment with losartan or PD123177 but not divalinal-Ang IV interfered with this effect of Ang II [61].

Effects of Ang II and Ang IV in the Passive Avoidance Task

Ang II impaired memory acquisition in rats, when injected at a dose of 5–50 fM into the CA1 area of the dorsal hippocampus, 90 min before the training trial in a passive avoidance setup [172]. This effect corresponded to the inhibitory action of Ang II on LTP in the dentate gyrus [136] and was similarly blocked by the AT₁ receptor antagonist losartan [172]. In the same setup, Ang II impaired memory consolidation when injected into the dorsal hippocampus up to 30 min after the training trial, and memory retrieval when injected 15 min before the testing trial [173,174]. The effects on memory consolidation and retrieval were observed when Ang II was administered at a dose of 0.05–0.5 nM and were blocked with the AT₂ receptor antagonist PD123319 but not with losartan [173,174] (Table 4).

When Ang II was injected into the lateral ventricle of rats, less consistent results were obtained. Depending on the dose and timing of administration, Ang II improved memory consolidation [175–177] and retrieval [165,178,179] or impaired memory consolidation [178], whereas in other studies, no effects were observed on memory acquisition [178], consolidation [3], or retrieval [3,170]. In one study, the time dependency of the effect of Ang II was illustrated by the observation that it improved memory retrieval when injected at a dose of 1 nmol into the lateral ventricle 15 min before the testing trial but had no effect when injected 5 min before the testing trial [170]. The facilitatory effect of Ang II on memory retrieval was blocked by the AT₁ receptor antagonists losartan and valsartan [165,179] (Table 4). This suggests that AT₁ receptor activation improves memory retrieval in the passive avoidance task. However, systemic administration of losartan in mice before the retrieval session improved memory retrieval and attenuated the scopolamine-induced deficit in the passive avoidance task, suggesting a deleterious effect of AT₁ receptor activation [180]. Similarly, chronic treatment with the AT₁ receptor antagonist E4177 improved passive avoidance memory retention in aged Dahl rats [181]. Species and strain differences may contribute to these observed discrepancies.

Ang IV, in contrast to Ang II, had no effect on memory consolidation or retrieval in the passive avoidance task when a similar dose was injected into the dorsal hippocampus [173,174]. Ang IV improved memory consolidation in the passive avoidance task when injected into the lateral ventricle after the training session [3,168,182] and enhanced memory retrieval when injected before the testing session [171] (Table 4). These effects were observed when Ang IV was administered at a typical

Table 4 Effects of Ang II and Ang IV on passive avoidance memory

Ligand	Administration	Timing	(Min)	Dose (nmol)	Effect	Receptor	Reference
Ang II	HPC	Pretraining	90	0.005–0.05	↓	AT ₁	[172]
		Posttraining	0–30	0.05–0.5	↓	AT ₂	[173]
		Pretesting	15	0.05–0.5	↓	AT ₂	[174]
	ICV	Pretraining	5	0.5–3	-	NA	[178]
		Posttraining	0	0.1–0.5	↑	ND	[175–177]
			5	0.5–3	↓	ND	[178]
			5	1	-	NA	[3]
		Pretesting	15	1	↑	AT ₁	[165,179]
			5	1–3	↑	ND	[178]
			5	1	-	NA	[3,170]
Ang IV	HPC	Posttraining	0–90	0.6	-	NA	[173]
		Pretesting	15	0.6	-	NA	[174]
	ICV	Posttraining	5	0.1–1	↑	AT ₄ /AT ₁ ?	[3,168,182]
		Pretesting	5–15	1	↑	ND	[3,171]

HP, intrahippocampal; ICV, intracerebroventricular; ↓, decreased memory performance; ↑, improved memory performance; -, no effect; NA, not applicable; ND, not determined.

dose of 1 nmol, but the involved receptor subtype is not clearly established. In one study, [Sar¹,Ile⁸]-Ang II impaired memory consolidation in the inhibitory avoidance task and blocked the memory-enhancing effect of Ang IV [182], suggesting that the effect on memory consolidation may be mediated through AT₁ or AT₂ receptors. However, Wright et al., demonstrated that administration of divalinal-Ang IV after training impaired memory consolidation in the passive avoidance task, thus suggesting a role for the AT₄ receptor [183]. Furthermore, it was demonstrated that i.c.v. injection of 1 nmol LVV-H7 attenuated the learning deficit observed for scopolamine-treated animals in the passive avoidance task [11]. Since LVV-H7 has no known affinity for the AT₁ or AT₂ receptor subtypes, a specific role for the AT₄ receptors was proposed [11].

Effects of Ang II and Ang IV in the Active Avoidance Task

Synaptic plasticity in the lateral amygdala is considered to be crucial for fear learning in the active avoidance task

[184]. Ang II has been demonstrated to suppress both LTP and LTD in the amygdala [129,130]. Nevertheless, several studies demonstrated the facilitatory action of Ang II on the acquisition of a multitrial active avoidance task when administered into the dorsal hippocampus or the lateral ventricle before the training session at a dose of 0.1–1 nM [165,179,185–188] (Table 5). Ang II injection into the lateral ventricle also improved memory consolidation when administered immediately after training at a dose of 0.1 nM [176,189]. This suggests that the effects of Ang II may not be directly mediated through the amygdala. The effect of i.c.v. injection of Ang II on memory acquisition could not be blocked by losartan [165] or valsartan [179] but was blocked by PD123319 [188]. Intriguingly, combined administration of losartan and PD123319 impaired acquisition of the active avoidance task [188], suggesting a role for endogenous angiotensins in active avoidance memory. Indeed, acute or chronic administration of the ACE-inhibitortrandopril attenuated the acquisition of the active avoidance task [190]. In contrast, in an earlier study, the nonselective angiotensin antagonist [Sar¹,Ile⁸]-Ang II produced an effect similar to that of Ang II [186]. As observed for Ang II, i.c.v. injection of

Table 5 Effects of Ang II and Ang IV on passive avoidance memory

Ligand	Administration	Timing	(Min)	Dose (nmol)	Effect	Receptor	Reference
Ang II	HPC	Pretraining	5	0.5	↑	ND	[185]
	ICV	Pretraining	15	0.1–1	↑	AT ₂	[165,179,186–188]
		Posttraining	0	0.1	↑	ND	[176,189]
Ang IV	ICV	Pretraining	15	1	↑	ND	[168–171,191]

HPC, intrahippocampal; ICV, intracerebroventricular; ↓, decreased memory performance; ↑, improved memory performance; ND, not determined.

Table 6 Effects of Ang II and Ang IV on novel object recognition memory

Ligand	Administration	Timing	(Min)	Dose (nmol)	Effect	Receptor	Reference
Ang II	ICV	Pretesting	15	1	↑	AT ₁ /AT ₂ ?	[165,179,187,192]
Ang IV	ICV	Pretesting	15	1	↑	ND	[168,169,171,191]

↑, improved memory performance; ND, not determined.

1 nmol Ang IV also enhanced the acquisition of a multitrial active avoidance task when given 15 min before training [168,169,171,191] (Table 5). However, the receptor subtype involved in this Ang IV effect was not determined.

Effects of Ang II and Ang IV in the Novel Object Recognition Task

In the novel object recognition task, which is not fear motivated but evaluates the rate of exploration of a novel object compared to a familiar object, injection of 1 nmol Ang II into the lateral ventricle, 15 min before testing, enhanced memory retrieval [165,179,187,192] (Table 6). This effect was abolished by pretreatment with losartan, valsartan, or CGP 42112A [165,179]. Similarly, Ang IV facilitated the memory retrieval in the novel object recognition task when injected at a dose of 1 nmol 15 min before training. The receptor subtype mediating this effect of Ang IV has not been determined [168,169,171,191] (Table 6).

Effects of Ang II and Ang IV in Spatial Memory Tasks

The effects of Ang II on spatial learning and memory are not clearly established. Ang II facilitated spatial memory consolidation in the food-rewarded T-maze spatial discrimination task when administered into the lateral ventricle at a dose of 1 nmol immediately after training, but did not affect memory retrieval when administered 15 min before testing [187]. In a foot shock-reinforced 6-chamber spatial maze [193] or Morris water maze setup [194], i.c.v. administration of the same dose of Ang II had no effect on spatial memory. While endogenous Ang II does not appear to be necessary for normal memory function [195], chronic ACE inhibition improved spatial memory in normal rats [196], in rats with scopolamine-induced memory deficits [197], and in Dahl salt-sensitive rats [181]. In normal rats, losartan and PD123319 did not affect spatial memory performance in the T-maze spontaneous alternation task [198]. However, losartan attenuated scopolamine-induced deficits in

the 12-arm radial maze [197], ethanol-induced deficits in the 8-arm radial maze [199] and the 8-arm radial maze deficit associated with Goldblatt-induced renal hypertension [200]. Similarly, the AT₁ receptor antagonist E4177 sustained spatial memory function in aged Dahl rats, even at a dose that did not affect blood pressure [181]. Taken together, these animal experiments suggest the involvement of endogenous Ang II in spatial memory deficits. ACE inhibitors similarly improved episodic memory irrespective of their blood pressure-lowering effects in hypertensive patients [201,202] and patients with a history of stroke or transient ischemic attack [203]. In very elderly hypertensive patients, chronic AT₁ receptor blockade by losartan, telmisartan, or valsartan improved episodic memory independent of antihypertensive effects [204–206]. Treatment with candesartan attenuated the decline in attention and episodic memory in elderly hypertensive patients [207], but another study reported no effect on general cognitive function in a controlled clinical trial on elderly hypertensive patients [208]. Since long-term treatment with candesartan increased circulating Ang IV levels in chronic renal failure patients [209], it is conceivable that some of the central effects of AT₁ receptor antagonists are indirectly mediated through Ang IV. Since angiotensin peptides do not effectively cross the blood–brain barrier, these effects may result from the action of Ang IV on neurons in the subfornical organ, organum vasculosum of the lamina terminalis, or area postrema, which lack a blood–brain barrier [210].

Consistent spatial learning and memory-improving effects have been described for AT₄ receptor ligands (Table 7). It was demonstrated that chronic i.c.v. infusion of Nle¹-Ang IV at a rate of 0.1–0.5 nmol/h enhanced the acquisition of the Morris water maze task in rats [7]. Importantly, i.c.v. infusion of the putative AT₄ antagonist divalinal-Ang IV at a rate of 0.5–5 nmol/h impaired the acquisition of spatial search strategies [7]. This finding suggests that the AT₄ receptor is required for spatial learning and memory. In one study, a single i.c.v. injection of 1 nmol Ang IV 15 min before the first trial of Morris water maze testing did not affect task performance [211]. However, in the Barnes circular maze, a single injection Nle¹-Ang IV or LVV-H7 into the lateral ventricle

Table 7 Effects of AT₄ ligands on spatial learning and memory

Ligand	Dose	Route	Task	Model	Effect	Reference
Nle ¹ -Ang IV	0.1–0.5 nmol/h: 7 days	ICV	MWM	NA	↑	[7]
Divalinal-Ang IV	0.5–5.0 nmol/h: 7 days	ICV	MWM	NA	↓	[7]
Ang IV	1 nmol: single injection	ICV	RAM	NA	↑	[171]
Ang IV	1 nmol: single injection	ICV	MWM	NA	-	[211]
Nle ¹ -Ang IV	0.1 nmol: single injection	ICV	BM	NA	↑	[70]
LVV-H7	0.1 nmol: single injection	ICV	BM	NA	↑	[70]
Norleucinal ¹ -Ang IV	1 nmol: 5 days	ICV	MWM	PP CUT	↑	[7]
Nle ¹ -Ang IV	0.01–1 nmol: 5 days	ICV	MWM	SCOP	↑	[8,9]
Nle ¹ -Ang IV	1 nmol: 5 days	ICV	MWM	MEC	↑	[10]
LVV-H7	1 nmol: 5 days	ICV	MWM	SCOP	↑	[11]

↑, improved memory performance; ↓, impaired memory performance; -, no effect; ICV, intracerebroventricular; MWM, Morris water maze; RAM, radial arm maze; BM, Barnes maze; PP CUT, perforant path lesion-induced memory deficit; SCOP, scopolamine-induced memory deficit; MEC, mecamylamine-induced memory deficit.

5 min before training on the first day of testing enhanced the spatial memory performance in rats [70]. Both Nle¹-Ang IV and LVV-H7 displayed a bell-shaped pharmacological profile with an optimal dose of 0.1 nmol, and a rapid effect on acquisition of the task was suggested [70]. This was supported by the observation that Ang IV facilitated spatial short-term memory in the radial arm maze when a similar dose was administered 15 min before the first test session [171].

Several studies demonstrated that putative AT₄ agonists improved Morris water maze performance in rat models for spatial learning and memory deficits. Typically, a dose in the range of 0.1–1 nmol was administered into the lateral ventricle, 15–20 min before each daily testing series. Nle¹-Ang IV reversed the spatial memory deficit induced by bilateral perforant path lesion [7], and LVV-H7 reversed the spatial memory deficit induced by scopolamine [11]. Similar studies reported that daily administration of Nle¹-Ang into the lateral ventricle reversed spatial memory deficits in the Morris water maze induced by the muscarinic receptor antagonist scopolamine or the nicotinic receptor antagonist mecamylamine [8–10]. However, Nle¹-Ang IV did not attenuate the spatial memory deficit induced by the combined administration of scopolamine and mecamylamine [10]. This suggests that the spatial memory-enhancing effect of Nle¹-Ang IV is dependent on a partially intact cholinergic system. In support of this hypothesis, it was demonstrated that both Nle¹-Ang IV and LVV-H7 facilitate K⁺-evoked acetylcholine release in hippocampal slices. These effects were attenuated by divalinal-Ang IV but not by the AT₁ and AT₂ receptor antagonists candesartan and PD123319 [69].

Potential Mechanisms for Facilitation of Memory by AT₄ Ligands

A role for the AT₁ or AT₂ receptor subtypes in the effects of Ang IV on active avoidance and object recognition memory can not be excluded. However, in passive avoidance and spatial memory, a role for the AT₄ binding site appears to be more clearly established. Several hypotheses have been proposed for the memory-enhancing properties of AT₄ ligands, as reviewed by Chai et al. [98]. Facilitation of glucose uptake into hippocampal neurons may contribute to the facilitatory effect of AT₄ ligands on spatial memory. The hippocampus is indeed an important target site of the memory-enhancing effects of glucose since intrahippocampal glucose administration enhanced spatial learning and memory in rats [148,212,213], and decreases in extracellular glucose levels were observed in the rat hippocampus during execution of a spatial memory task [214].

Alternatively, AT₄ ligands may exert their learning and memory-enhancing effects indirectly by inhibiting the catalytic activity of IRAP and extending the half-life of its neuropeptide substrates. Several memory-modulating neuropeptides were demonstrated as IRAP substrates *in vitro* [81–84,215]. Arg-vasopressin has a well-recognized role in social recognition and spatial memory, acting mainly on the vasopressin V1a receptor [216–221]. Dynorphin A and neurokinin A improved scopolamine-induced spatial short-term memory deficits in rats [222–224]. The role of other potential IRAP substrates such as oxytocin in memory-related behaviors is less clearly defined [221]. However, oxytocin was demonstrated to improve spatial long-term but not short-term memory in female mice [142]. Somatostatin-14 alleviated short-term

memory impairments induced by muscarinic receptor antagonists in rats [225] but interfered with the flexible use of spatial memory in mice [226], and intrahippocampal dynorphin B injection impaired spatial learning in rats [227].

Several objections to the enzyme inhibition hypothesis were discussed by Wright *et al.* (2008). Intriguingly, the putative AT₄ receptor antagonist divalinal-Ang IV impaired spatial learning in rats [7], blocked the effects of Ang IV on long-term potentiation [96] and the effects of Ang IV and LVV-H7 on acetylcholine release in rat hippocampal slices [69], and has also been characterized as a competitive inhibitor of IRAP, albeit with a relatively low affinity [84].

Administration of HGF or its mRNA into the lateral ventricle improved the spatial learning and memory deficit induced by cerebral ischemia in rats [228,229]. HGF gene transfer alleviated amyloid- β -induced impairment of spatial short-term memory and long-term memory [230]. This beneficial effect was associated with a recovery of vessel density, decrease of oxidative stress, upregulation of brain-derived neurotrophic factor, and synaptic enhancement. Taken together, this suggests that c-MET is an interesting target for the treatment of Alzheimer's disease and cerebrovascular diseases such as cerebral infarct and vascular dementia. However, at present, there is yet no evidence for the involvement of c-MET in the cognitive effects of AT₄ ligands.

Effects of Ang II and Ang IV in Animal Seizure Models

Plasticity changes are recognized factors in the evolution of epilepsy. The hippocampal formation and amygdala are highly susceptible to plasticity changes and play key roles in epileptogenesis [231,232]. Kindling by electrical stimulation of the perforant path and lateral amygdala have been used as animal models for temporal lobe epilepsy [233–235] and share a common molecular mechanism with LTP [126,236]. The effect of Ang II and Ang IV on synaptic efficacy in the hippocampus and amygdala are therefore indicative of a possible modulatory action on epileptogenesis. Ang IV had antiepileptogenic effects during pentylentetrazole (PTZ) kindling in mice [14], which shares a common mechanism with electrical kindling [237]. Furthermore, both Ang II and Ang IV suppressed seizures in PTZ-kindled mice [13,238–240]. This supports the notion that both peptides may share common effects on neuronal excitability and synaptic plasticity in brain areas involved in seizure susceptibility.

Effects of Ang II in Animal Seizure Models

I.c.v. injection of Ang II reduced the intensity of seizures in acute chemoconvulsant mice models such as intraperitoneal (i.p.) injection of biccuculine, picrotoxin, or PTZ [241,242] and reduced the seizure threshold for intravenous (i.v.) infusion of PTZ [243–245]. Ang II reduced seizure intensity in mice kindled by repeated i.p. injections of PTZ and suppressed the progression of kindling induced by repeated PTZ administration [238–240]. These anticonvulsive and antiepileptogenic effects were typically observed when 1 nmol Ang II was administered 15 min before PTZ treatment. The increase in PTZ seizure threshold induced by Ang II was blocked by i.c.v. administration of the AT₁ receptor antagonist losartan but not by the AT₂ receptor antagonist PD123319 [244,245]. Surprisingly, combined i.c.v. injection of an ineffective dose of Ang II with either losartan or PD123319 decreased seizure intensity in PTZ-kindled mice [239]. The authors suggested that losartan and PD123319 may act as partial agonists of the AT₁ and AT₂ receptor subtypes, respectively. However, taken together, these studies indicate a role for the AT₁ receptor subtype in the effects of Ang II on seizure susceptibility. Furthermore, in the hippocampus of patients with temporal lobe epilepsy, the immunoexpression of both AT₁ and AT₂ receptors was upregulated, and an increase in the mRNA expression was demonstrated for AT₁ receptors but not for AT₂ receptors [246]. This finding supports the notion that the AT₁ and possibly the AT₂ receptor subtypes may be involved in the physiopathology of temporal lobe epilepsy.

The adenosine A₁ receptor antagonist 8-(p-sulphophenyl)-theophylline [244], the α_1 -adrenoreceptor antagonist prazosine, the α_2 -adrenoreceptor antagonist yohimbine [245], and the mixed dopamine D₁/D₂ receptor antagonist pimozide [243] suppressed the effects of Ang II on seizure susceptibility, suggesting modulation of signaling mechanisms downstream of adenosine, noradrenaline, and dopamine, as reviewed in detail by Tchekalarova *et al.* [247]. Indeed, endogenous adenosine and serotonin have been described to have antiepileptic properties [248–250]. In addition, an allosteric modulation of GABA receptors by Ang II was proposed [251].

Effects of Ang IV in Animal Seizure Models

Ang IV injection into the lateral ventricle increased the PTZ seizure threshold and reduced the seizure intensity in mice kindled by repeated i.p. injections of PTZ [13]. These effects were observed when Ang IV was administered at a dose of 0.1–1 nmol 15 min before PTZ administration. Furthermore, i.p. injection of 0.2 mg/kg Ang IV was

protective in mice against PTZ-induced kindling. The receptor subtype involved in the anticonvulsive and antiepileptogenic effects of Ang IV was not investigated. However, an area-specific increase in dopamine D1 receptor density in the rostral neostriatum was observed after i.p. injection of Ang IV [14]. This suggests that Ang IV may modulate seizure susceptibility through dopaminergic modulation in the basal ganglia. The anticonvulsive effects of Ang IV were reversed by the A₁ adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine, suggesting a modulatory action on adenosine signaling [13].

Infusion of Ang IV into the lateral ventricle at a rate of 10 nmol/h protected rats against pilocarpine-induced limbic seizures. This protective effect was associated with a slow increase of hippocampal dopamine and serotonin levels and could be blocked by the somatostatin SST₂ receptor antagonist cyanamid 1554806. i.c.v. infusion of somatostatin-14 similarly protected against pilocarpine-induced seizures and increased hippocampal dopamine and serotonin levels [12]. Increased hippocampal dopamine and serotonin levels were previously demonstrated to prevent pilocarpine-induced seizures [252,253]. These results suggest that Ang IV may exert an anticonvulsive effect via inhibition of IRAP, resulting in an increase of the anticonvulsive neuropeptide somatostatin-14.

Potential Anticonvulsive Mechanisms of AT₄ Ligands

The anticonvulsive effect of Ang IV in the focal pilocarpine model is independent of AT₁ receptor activation and may be mediated through inhibition of IRAP and degradation of its substrate somatostatin-14. Indeed, somatostatin-14 is an inhibitory modulator of hippocampal circuitries and is critically involved in temporal lobe epilepsy [254–257]. Somatostatin-14 exerts a robust antiepileptic activity *in vitro* and *in vivo* [257]. Somatostatin knockout mice display a higher sensitivity to kainate-induced limbic seizures but a similar sensitivity to kindling by electrical stimulation of the perforant path [255]. Besides somatostatin, other IRAP substrates are involved in seizures. Endorphins such as dynorphin A may control hippocampal excitability and protect against hippocampal seizures through activation of κ -opioid receptors [258]. Tachykinins such as neurokinin A are believed to be critical for the control of hippocampal excitability and hippocampal seizures, as evidenced by the resistance of preprotachykinin A gene null mice to kainate-induced limbic seizures [259]. Despite the similar actions of Ang II and Ang IV on PTZ-induced seizures, the AT₁ receptor antagonist candesartan was unable to block the effect of Ang IV on pilocarpine-induced seizures [12].

The role of glucose in epileptic seizures is complex. Temporal lobe epilepsy is associated with impaired hippocampal glucose metabolism independently of neuronal loss and may be a result of reversible neuronal dysfunction [260,261]. Mutation of the GLUT₁ glucose transporter, which is expressed at high levels in endothelial cells composing the blood–brain barrier, results in an encephalopathy associated with low brain glucose levels and epileptic seizures [262,263]. Therefore, it could be proposed that AT₄ ligands may ameliorate the neuronal metabolic dysfunction by facilitation of neuronal glucose uptake through the glucose transporter GLUT₄. However, the glycolysis inhibitor 2-deoxy-D-glucose exhibited anticonvulsant and antiepileptic properties in the electrical kindling model of temporal lobe epilepsy in rats, demonstrating that blocking glucose utilization may suppress seizure activity [264]. This concept was proposed as the scientific basis for the strict ketogenic diet that has been used successfully to treat refractory epilepsy.

Interestingly, mice with a targeted mutation of the urokinase plasminogen activator receptor, a key component in the HGF activation, display a decreased expression of HGF associated with marked decrease in cortical GABA interneurons and exhibited spontaneous seizures and an increased sensitivity to pharmacologically induced seizures [265,266]. It is however unclear how activation of c-MET may account for the acute anticonvulsive and antiepileptogenic effects of Ang IV in adult mice and rats.

Conclusion and Future Perspectives

Exogenous Ang II affects neuronal excitability and plasticity, learning, memory, and epileptic seizures. However, different effects have been observed depending on the dose or concentration of the peptide, site, and timing of administration and the used task or animal model. Endogenous Ang II may not be necessary for normal memory function, but blocking its formation by ACE or its action on AT₁ receptors improved memory function in animal models of amnesia and hypertension. These results from animal experiments have been confirmed in several clinical studies. ACE inhibitors improved cognition in patients with stroke or hypertension and AT₁ antagonists enhanced episodic memory in elderly hypertensive patients irrespective of their antihypertensive effects. Indeed, since hypertension is a major risk factor in cognitive impairment, it is important to consider the peripheral effects of these drugs [267]. The role of endogenous Ang II in animal seizure models is less verified. The observation that AT₁ and AT₂ receptors are upregulated in

patients with temporal lobe epilepsy warrants further investigation of the role of angiotensin peptides in epilepsy.

Ang IV and its binding site became a focus of interest because of the beneficiary effects of exogenous Ang IV in animal models for cognitive impairment and epileptic seizures. However, the nature of this binding site remains unresolved. Future experiments using IRAP or c-MET knockout animals and selective IRAP inhibitors or c-MET antagonists may help to answer this challenging question and provide insights into the role of endogenous Ang IV or other AT₄ ligands in cognitive disorders and epilepsy.

Conflict of Interest

The authors have no conflicts of interest.

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