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The Development of Small Molecule Angiotensin IV-Based Analogs to Treat Depression

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Abstract: Major depression is a common form of mental disorder affecting approximately 15% of the population at least once during lifetime. The etiology of depression is complex with potential contributions from central and peripheral systemic factors, and several CNS impacting diseases. Presently employed antidepressant medications are poorly responded to by upwards of 50% of patients and typically require weeks of treatment to be effective. Recent post-mortem brain scans indicate significant volume reductions in two limbic brain structures, the hippocampus and prefrontal cortex of depressed patients. These findings focus attention on hippocampal plasticity in the neuropathology of depression and the possible dysfunction of several important processes including neurogenesis, synaptogenesis, and contributions by neurotrophic growth factors. The hepatocyte growth factor (HGF)/c-Met receptor system is a powerful mediator of synaptogenesis and neurogenesis, and if adequately activated may serve to counter the neuropathology of depression. The brain renin-angiotensin system (RAS) interacts with the HGF/c-Met system and plays a major role in responding to stress and the pathophysiology of depression. We have developed an angiotensin IV-based small molecule designed to activate the HGF/c-Met receptor system in order to provide neuroprotection, synaptogenesis, and neurogenesis in the hippocampus and prefrontal cortex. This analog may be efficacious in treating the neuropathology of depression.

Keywords: Depression, Angiotensin II, Angiotensin IV, AT_4 Receptor, Hepatocyte Growth Factor, C-Met Receptor, Dihexa

1. Introduction

Major depression is among the most common forms of mental disorder affecting approximately 15% of the population at least once during lifetime [1]. At any given time depression is experienced by approximately 2% of children and 5% of adolescents [2]. The likelihood of depression increases with age particularly among those with functional disabilities, and/or physical and cognitive illness [3-5]. Community/residence-seniors have reported the prevalence of major depression estimated at 10% [4-5]. The pathophysiology of adult depression is complex with contributing factors that may include CNS and peripheral systemic factors, while Alzheimer's disease, Parkinson's disease, and stroke are recognized risk factors [6-8]. Cancer, cardiovascular disease, metabolic and endocrine dysfunction are also often associated with depression [9,10]. Identifying reliable biomarkers of depression has been challenging [11]. Many hypotheses have been posited to explain adulthood depression including alterations in glucocorticoid regulation and related stress hormones [12], insulin resistance [13], inflammatory chemokines and cytokines [14], and various trophic factors that are stimulated with injury, illness and other stressors [15]. Along these lines accumulating evidence suggests that depression accompanying diabetes mellitus significantly increases pro-inflammatory mechanisms and a loss of hippocampal neuroplasticity [16-18]. The antidepressant classes of medication presently available (5-hydroxytryptamine and norepinephrine-selective reuptake inhibitors) lack effectiveness in upwards of 50% of patients and typically require weeks of treatment to be effective [19].

Recent post-mortem brain scans of depressed patients evidenced significant reductions in the volume of limbic brain structures most notably in the hippocampus (Hip) and prefrontal cortex (PFC) [20,21]. Of particular importance exposure to stress has been linked with neuronal atrophy and loss of glia in both structures [22,23]. The formation of new

neurons in the adult brain (neurogenesis) is known to occur in the subgranular zone of the dentate gyrus of the Hip and subventricular zone of the lateral ventricles [24,25]. Neural stem cells in these structures are capable of dividing asymmetrically to form a daughter stem cell and a rapid multiplying progenitor cell. If appropriately stimulated these progenitor cells mature into neurons that integrate into functional neuronal networks [26,27]. Chronic stress-induced depression decreases neurogenesis; however treatment with antidepressant drugs may reverse this process [22,28]. These observations point to the involvement of dysfunctional hippocampal plasticity in the neuropathology of depression, with particular focus on neurotrophic growth factors. The "neurotrophic hypothesis" of depression suggests that depression results from decreased neurotrophic growth factor activity causing atrophy of neurons in the Hip and PFC coupled with decreased neurogenesis and loss of glia. It has been hypothesized that treatment with antidepressant drugs interferes with and/or blocks neurotrophic factor deficits thus reversing atrophy [22-25]. The neurotrophic growth factors thus far linked with depression include vascular endothelial growth factor (VEGF), fibroblast growth factor-2, and insulin-like growth factor (IGF-1), with particular interest in brain-derived neurotrophic factor (BDNF) [29-32]; BDNF appears to be necessary for a positive response to treatment with antidepressant drugs [22,33]; however, preclinical results concerning the role of BDNF depletion in the etiology of depression are less consistent. BDNF-deletion mutant mice generally reveal normal behavior when tested for depression although conditional female mutant mice have been reported to show increased immobility during forced swim testing [34]. The use of RNA interference to knock down BDNF expression in hippocampal substructures results in depression as measured using forced swim and sucrose preference tasks [35].

This review focuses on a new target of potential importance as a treatment of depression, the brain renin-angiotensin system (RAS), and the recent discovery that it acts via the hepatocyte growth factor (HGF)/ tyrosine kinase c-Met receptor system (reviewed in [36,37]). The HGF/c-Met receptor system functions as a critical survival mechanism for motor, internuncial and sensory neurons and a subset of root ganglion neurons [38,39]. This relationship between the RAS and HGF/c-Met systems offers clinically relevant possibilities that small angiotensin-based molecules can be designed to act as agonists at the HGF/c-Met complex in place of large protein ligands. The next sections provide descriptions of the RAS and HGF systems, information concerning their interaction, and the involvement of the RAS and HGF systems in stress and depression. We conclude with details concerning a newly developed angiotensin IV (AngIV)-based small molecule that activates the HGF/c-Met receptor system, promotes synaptogenesis, and offers neuroprotection thus encouraging neuron survival.

2. The Brain Renin-Angiotensin System

The RAS regulates systemic blood pressure and body water balance, activates sympathetic pathways, and exerts control over vasopressin and oxytocin synthesis and release [40,41]. These functions are mediated, in part, by an independent brain RAS complete with the necessary components including angiotensinogen, renin, angiotensin converting enzyme (ACE), angiotensin ligands, and receptor proteins ([42,43] Figure 1). Following the discovery of this independent brain RAS separate from the peripheral system three brain angiotensin receptor subtypes were identified. The first two, AT_1 and AT_2, are G-protein coupled and have been well described in previous review papers [36,40,41,44]. Several years ago members of our laboratory discovered a third subtype, AT_4, which is a major focus of this review.

Figure 1

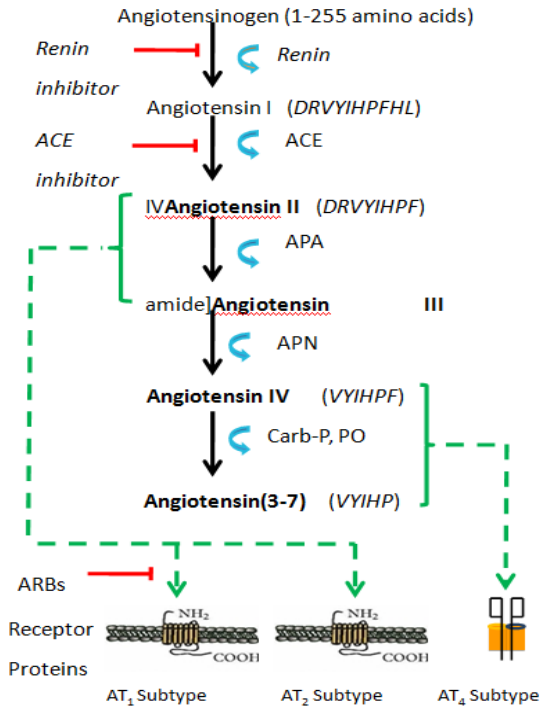

Figure 1. *The renin-angiotensin pathway indicating the biologically active ligands (bold), enzymes, receptors and inhibitors involved in angiotensin mediated physiologies and behaviors. Both angiotensin II and III bind to the AT_1 and AT_2 receptor subtypes. Angiotensin IV and angiotensin (3-7) bind at the AT_4 subtype. Abbreviations: ACE, angiotensin converting enzyme; APA, aminopeptidase A; APN, aminopeptidase N; ARBs, angiotensin receptor blockers; Carb-P, carboxypeptidase P; PO, propyl oligopeptidase.*

The AT_1 subtype is localized in high densities within the anterior pituitary, area postrema, lateral geniculate body, inferior olivary nucleus, median eminence, nucleus of the solitary tract, the anterior ventral third ventricle region, paraventricular, preoptic and supraoptic nuclei of the hypothalamus, subfornical organ, ventral tegmental area, caudate putamen, cerebellum, striatum, and substantia nigra. The AT_1 receptor plays a critical role in promoting oxidative stress which in turn encourages neurodegeneration that impacts CNS neurons [45,46].

The highest densities of the AT_2 receptor site are found in the amygdala, medial geniculate body, habenula, hypoglossal nucleus, inferior colliculus, inferior olivary

nucleus, locus coeruleus, striatum, thalamus, ventral tegmental area, caudate putamen, cerebellum, globus pallidus, and substantia nigra. There is recent evidence suggesting that activation of the AT_2 receptor may offer neuroprotection [47]. The roles of the AT_1 and AT_2 subtypes in neurodegeneration and neuroprotection will be discussed in a subsequent section.

The AT_4 receptor is distributed within a number of brain structures with notably high concentrations in the anterior pituitary, cerebral cortex, lateral geniculate body, habenula, inferior olivary nucleus, nucleus basalis of Meynert, periaqueductal gray, piriform cortex, superior colliculus, thalamus, and ventral tegmental area, caudate putamen, cerebellum, globus pallidus, nucleus accumbens, red nucleus, substantia nigra and striatum. Of particular interest AT_4 receptors, which represent the molecular target of AngIV, are prominently represented in the Hip and PFC. Although the brain distribution of AngIV is not available, the locations of aminopeptidase A (AP-A, an aminopeptidase that converts the octapeptide AngII to the heptapeptide AngIII) and aminopeptidase N (AP-N, an aminopeptidase that converts AngIII to the hexapeptide AngIV) are suggestive given their likely co-localization with AngIV (see Figure 1). Both AP-A and AP-N have been localized to the plasma membrane of pericytes suggesting that AngIV is found in the extracellular space surrounding microvessels in the brain [48]. In support of this notion exogenous administration of Ang IV has been shown to increase cerebral microcirculation [49-51]. Of relevance, Lanckmans and colleagues [52,53] measured AngIV in the striatum using microdialysis coupled with a sensitive liquid chromatography mass spectrometry system. Shortly following probe insertion the levels of AngIV dropped below the detection limit of 50 pM. This was interpreted to suggest an intracellular presence for AngIV. This notion is supported by several reports indicating that within neurons AngII is converted to AngIV (80%), with smaller fractions of AngIII, Ang(1-7), and Ang(1-6) (reviewed in [54]). Thus the AT_4 receptor, co-localized with AngIV, is prominently represented in the Hip and PFC, two structures implicated in major depression.

3. The Brain Hepatocyte Growth Factor/c-Met Receptor System

As indicated by its name HGF was originally isolated from liver and has been shown to promote liver regeneration [55]. HGF is a glycoprotein also known as "scatter factor" that acts as a potent mitogenic, morphogenic, and motogenic growth factor [56]. Some years ago Bottaro and colleagues [57] identified the Type 1 tyrosine kinase receptor c-Met as the receptor for HGF. The c-Met receptor protein is made up of disulfide bond-linked alpha (45 kDA) and beta (145 kDa) subunits (Figure 2 [58]). The alpha-chain is extracellular while the beta-chain is transmembrane. HGF dimerization precedes binding to the c-Met receptor which then undergoes phosphorylation. Once phosphorylated the tyrosine residues of the beta subunit serve as docking sites for downstream signaling mediators including the extracellular signal-regulated kinase (ERK) and the phosphatidylinositol-3-kinase (P13K) pathways [59]. This HGF/c-Met signaling is regulated by the activator hepatocyte growth factor A (HGFA) and its inhibitor, HGFAI. HGFA is a protease that acts on the precursor protein and produces active HGF. In contrast HGFAI blocks the activation of HGFA [60]. c-Met has been shown to play a role in multiple types of cancer (reviewed in [61]), blunt neurodegenerative changes [62], facilitate long-term potentiation (LTP [63]), contribute to learning and memory consolidation [64,65], and may play a role in Alzheimer's and Parkinson's diseases [66,67]. Also, inactivation of c-Met in the embryonic proliferative zones of mice results in an increase in parvalbumin-expressing cells in the dentate gyrus, a loss of these cells in the CA3 field, with an overall loss of calretinin-expressing cells throughout the Hip [68]. These results highlight the importance of c-Met with regard to appropriate hippocampal development. Several researchers have suggested the use of HGF as a therapeutic agent for amyotrophic lateral sclerosis, ischemia-stroke [64,69], neuroimmune [39,70] and neurodegenerative diseases [71], and to encourage neuron survival [72,73].

Figure 2

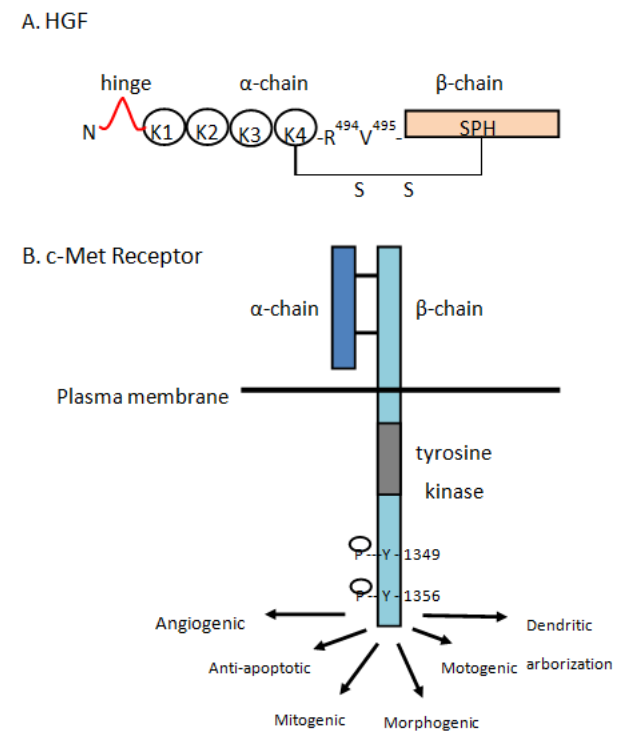

Figure 2. *A. Structure of hepatocyte growth factor (HGF) consisting of a α-chain (69 kDa) that includes four Kringle domains and a β-chain (43 kDa) plus a serine proteinase homology (SPH) domain, linked by disulfide bonds (S). B. Structure and basic functions of the c-Met receptor consisting of a α-chain (50 kDa) and a β-chain (140 kDa) linked by disulfide bonds. HGF binds to c-Met resulting in tyrosine phosphorylation leading to the activation of a number of biological functions.*

4. Interaction Between Angiotensin IV and the HGF/c-Met System

Several years ago members of our laboratory set about identifying a molecular target with structural homology to angiotensin IV and physiological functions in agreement with those identified for the AngIV/AT_4 receptor system. We discovered a partial match with the protein angiostatin, and the related plasminogen family member HGF. We noted that the functions associated with the HGF/c-Met system overlap extensively with those mediated by the AngIV/AT_4 system and include facilitated hippocampal long-term potentiation (LTP), calcium signaling, memory consolidation, augmented neurite outgrowth, dendritic arborization, facilitation of cerebral blood flow and cerebroprotection, seizure protection, and augmented wound healing (reviewed in [36,37]). This prompted the hypothesis that AngIV analogs may exert their activity via the HGF/c-Met system. Recently we reported that the AT_4 receptor antagonist Norleual-AngIV inhibited HGF binding to c-Met and HGF-dependent signaling, proliferation, invasion, and scattering [74]. The mechanism of action regarding Norleual-AngIV's ability to act as a c-Met receptor antagonist is by inhibiting the dimerization of HGF which is a prerequisite to c-Met receptor activation [75,76]. These results strongly suggest that the biological effects of endogenous AngIV, and AngIV analogs, are mediated through the HGF/c-Met system.

Subsequent research findings relevant to the hypothesis that the AngIV/AT_4 receptor system acts via HGF/c-Met include the following: 1) As mentioned earlier, in contrast with the AT_1 and AT_2 receptor subtypes heavy brain distributions of the AT_4 receptor reside in structures implicated in depressive disorders including neocortex, piriform cortex, hippocampus, and nucleus basalis of Meynert, consistent with expectations concerning brain locations for a receptor acting as a mediator of cognitive processing [77-80]. Partial determination of brain c-Met receptor distributions agree with this pattern [81,82]. 2) The AT_4 receptor subtype's ability to facilitate LTP, separate from NMDA-dependent LTP, suggests a nonglutamatergic signaling pathway [83] and agree with such findings concerning the HGF/c-Met system [63]. 3) The finding that facilitation of the AT_4 receptor subtype results in increased internalization of calcium via at least three different calcium channels, suggests a rapid and salient cell signaling event [83] and agrees with the observation that HGF-induced responses also depend upon the internalization of calcium [84]. 4) Conversion of AngII to AngIV appears to be necessary for AngII-induced DA release in the striatum [85], and acetylcholine release in the Hip [86] indicating the relatively greater importance of AngIV over AngII with regard to stimulating neurotransmitter release. 5) The coupling of increased neural intracellular calcium with matrix metalloproteinases released into the extracellular space suggests a neural plasticity function [87,88]. And 6) Recent neural imaging work completed in our laboratory indicates that the AT_4 receptor agonist Nle^1-AngIV (Figure 3) stimulates dendritic spine numbers and size in the Hip, as well as overall dendritic arborization suggesting a plausible mechanism to explain the ability of these molecules to enhance synaptic plasticity and connectivity among neurons [89]. Additionally, Nle^1-AngIV was found to be significantly more potent than BDNF in stimulating spine numbers and size. In agreement HGF has been shown to increase dendritic arborization in cultured hippocampal neurons [84].

Figure 3

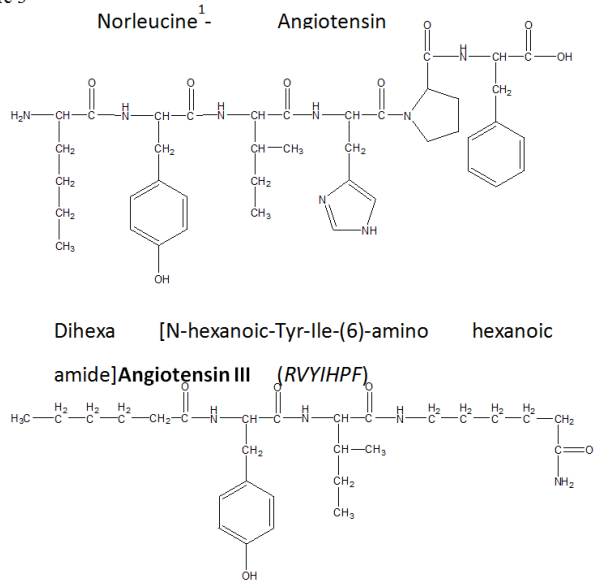

Figure 3. *Chemical structures of Nle^1-AngIV (Norleucine-YIHPF) and Dihexa (N-hexanoic-YI-(6) amino hexanoic amide).*

5. Stress, Depression and the RAS

Behavioral and physiological responses to acute stress, threat and danger are reasonably fixed and programed to promote survival. Thus, a rather elaborate neural circuitry is activated to modulate fear-related behaviors designed to protect the individual from damage while fleeing, defending or initiating attack. During acute stress cognitive repertoires are limited resulting in a fearful behavioral mode that appears to be under the control of the mesolimbic dopaminergic reward system [90,91]. Physiological responses include increased heart rate and blood pressure thus providing additional oxygenated blood to the brain and muscles groups; while corticotropin releasing hormone is released into the cerebroventricular system resulting in heightened arousal. This is accompanied by a succession of regulatory behaviors such as eating, drinking, sexual behaviors, sleeping, etc. [92,93]. Exposure to stress/threat is associated with a proinflammatory state that readies the immune system for possible injury. Elevated stress mediators include cortisol and cytokines that trigger insulin resistance thus raising plasma glucose levels. This mechanism is designed to benefit structures that are not dependent on insulin for glucose transport such as the brain and immune system [94]. Such a response to stress also promotes a procoagulant state in order to combat possible hemorrhage. Thus, plasminogen activator

inhibitor-1 is released from visceral fat cells in order to inhibit plasminogen accompanied by the release of fibrinogen.

During chronic stress and major depression the dopaminergic reward system is inhibited producing a state of anhedonia. A proinflammatory state is activated accompanied by a significant reduction in neuroplasticity and neurogenesis [95,96]. The RAS plays a prominent role in mediating these effects [97]. In particular AngII binding at the AT_1 receptor subtype promotes nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidases, a significant source of reactive oxygen species (ROS) [98,99]. Such activation of the AT_1 receptor also results in the stimulation of the NF-kB signal transduction pathway facilitating the synthesis of chemokines, cytokines, and adhesion molecules, all important in the migration of inflammatory cells into regions of tissue injury [100]. Given the above reports it follows that if AngII activation of the AT_1 receptor subtype results in facilitation of the NADPH oxidase complex and thus formation of free radicals, then blockade of the AT_1 receptor should serve a protective function. This appears to be the case [101,102]. Treatment with AT_1 receptor antagonists, known as angiotensin receptor blockers (ARBs), protects DA neurons in both 6-hydroxydopamine (6-OHDA) [90-94], and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models [103,104]. Specifically, ARBs have been shown to reduce the formation of NADPH oxidase-derived reactive oxygen species following administration of 6-OHDA [105]. Further, treatment with ACE inhibitors has been shown to offer protection against the loss of DA neurons in MPTP [106] and 6-OHDA animal models [107]. The likely mechanism underlying this ACE inhibitor-induced protection is a reduction in the synthesis of AngII acting at the AT_1 receptor subtype (reviewed in [108]).

As mentioned earlier AngII activation of the AT_2 receptor subtype has been shown to offer neuroprotection. The AT_2 receptor subtype is present in several fetal tissues including uterus, ovary, adrenal gland, heart, vascular endothelium, kidney and brain (particularly neocortex and hippocampus) [40,109-111]. As development progresses the expression of the AT_2 receptor decreases. It appears that adult mammalian brain levels of this receptor are reasonably low [41,112]. The AT_2 receptor has been linked with cell proliferation, differentiation, and tissue regeneration [113,114]. Results from a study utilizing mesencephalic precursor cells indicated that AngII, acting at the AT_2 receptor, facilitated differentiation of precursor cells into DA neurons [115]. Along these lines, activation of the AT_2 receptor has been shown to inhibit NADPH oxidase activation [47,116]. However, Rodriguez-Pallares et al. [117] found that AngII treatment of 6-OHDA lesioned adult rats increased neuron cell death. This could be due to the much greater relative numbers of brain AT_1 receptors, as compared with AT_2 receptors, such that the beneficial effects of AT_2 receptor activation was overwhelmed by AT_1 activation. For a thoughtful and detailed review of the literature concerned with the influence of acute and chronic stressors on the CNS and immunity system as related to depression the reader is referred to Gold [118].

6. Synaptogenesis and the RAS-HGF/c-Met System

As discussed earlier AT_1 receptor blockade has a neuroprotective effect [101,103]. Less obvious is the likelihood that AT_1 receptor blockade results in accumulating levels of AngII which is converted to AngIII and then to AngIV. Thus, an alternative explanation of these AT_1 receptor antagonist findings is that increases in endogenous AngIV levels facilitate activation of the HGF/c-Met receptor system resulting in neuroprotection of neurons. In this way AngIV may act in combination with AT_1 receptor blockade to protect neurons. Our laboratory has offered evidence that AngIV, and AngIV analogs, facilitate HGF/c-Met activity [89]. Support for this claim is presented in several recent reports. First we found that the action of AT_4 receptor antagonists depends on inhibiting the HGF/c-Met receptor system by binding to and blocking HGF dimerization [74,75]. In contrast, AT_4 receptor agonists facilitate synaptogenesis by acting as mimics of the HGF dimerization domain (see Figure 2 hinge region) [76]). This work has culminated in the synthesis of a small molecule AT_4 receptor agonist or active metabolite capable of penetrating the blood-brain barrier (BBB) and facilitating cognitive processing presumably by increasing synaptogenesis. This small molecule (MM-201, named Dihexa) has a Kd for HGF ≈ 65 picomolar [81]. The AngIV-HGF/c-Met interaction could explain earlier reports indicating that activation of the AT_4 receptor facilitated cerebral blood flow and neuroprotection [49,119,120].

In agreement with the above findings HGF has been shown to positively impact ischemic-induced injuries such as cardiac [121] and hind limb ischemia [122], and reduce the infarct volume of stroke [123]. HGF has also been shown to eliminate Hip neuronal cell loss in transient global cerebral ischemic gerbils [69], and transient focal ischemic rats [124]. Date and colleagues [64] have reported HGF-induced improvements in escape latencies by microsphere embolism-cerebral ischemic rats as measured using a circular water maze task. These authors noted reduced damage to cerebral endothelial cells in ischemic animals treated with HGF. Shimamura et al. [62] have shown that over-expression of HGF following permanent middle cerebral artery occlusion resulted in significant recovery of performance in the Morris water maze and passive avoidance conditioning tasks. Treatment with HGF was also found to increase the number of arteries in the neocortex some 50 days following the onset of ischemia.

As a means of better understanding how AT_4 receptor agonists and antagonists modify synaptic plasticity members of our group evaluated their influence on hippocampal LTP. We determined that the application of Nle^1-AngIV significantly facilitated LTP in the CA1 field of hippocampal slices [49]; while both AngIV and Nle^1AngIV enhanced LTP

in the dentate gyrus *in vivo* [125]. Pretreatment with the specific AT_4 receptor antagonist Divalinal-AngIV prior to tetanization significantly disrupted the maintenance phase of LTP. Nle^1-AngIV facilitation of LTP was shown to be dependent upon increased intracellular calcium via L- and T-type voltage-dependent calcium channels [83]. The ability of these agonists to promote Ca^{2+} entry, particularly via L-type channels, suggested the potential mechanism of altered dendritic arborization [126]. We next directly examined the ability of AT_4 agonists to facilitate dendritic arborization in disassociated rat hippocampal neurons labeled with mRFP-bactin to visualize the cytoskeleton, including the spines. Quantitative analysis from neurons exposed to Nle^1-AngIV for 5 days indicated an increased number of dendritic spines per dendrite, accompanied by a significant expansion of dendritic arborization [89]. The above observations support the hypothesis that the primary mechanism underlying memory facilitation by AngIV (and its analogs) is the ability to enhance synaptic communication and neural activity.

These Nle^1-AngIV-induced increases in dendritic arborization are consistent with the hypothesis that AT_4 receptor ligands alter HGF docking at the c-Met receptor. There are several reports indicating that HGF and c-Met are neuronally expressed in several brain structures including neocortex and Hip, and appear in high densities at excitatory synapses within the Hip [82]. Activation of the c-Met receptor by HGF promotes neurite outgrowth [127] and dendritic branching by cortical neurons in slice cultures [128]. The complexity of the dendritic branching could be attenuated with anti-HGF antibodies. Tyndall and colleagues [84] reported that HGF increased the size and complexity of dendritic arborization in dissociated Hip neurons in culture. This facilitation could be blocked by pretreatment with the NMDA receptor antagonist, DL-2-amino-5-phosphonopentanoic acid (APV). It was further determined that this HGF effect was dependent upon elevations in intracellular calcium and accompanying increases in autophosphorylation of CaMKII. These results suggest that calcium-dependent processing underlies HGF's ability to increase dendritic arborization, and are consistent with our findings indicating increased hippocampal neuronal intracellular calcium with Nle^1-AngIV treatment and facilitated hippocampal dendritic arborization. Pretreatment of cultured hippocampal neurons with an AT_4 receptor antagonist inhibited this Nle^1-AngIV-induced arborization [89].

In sum, these results indicate a role for the HGF/c-Met receptor system in cerebroprotection and are consistent with the notion that AngIV increases blood flow by a NO-dependent mechanism [50]. In support of this hypothesis Faure et al. [129] have reported that increasing doses of AngIV via the internal carotid artery significantly decreased mortality and cerebral infarct size in rats twenty-four hours following embolic stroke due to the intracarotid injection of calibrated microspheres. Pretreatment with the specific AT_4 receptor antagonist Divalinal-AngIV, or Nω-nitro-L-arginine methyl ester (L-NAME), abolished this protective effect. Sequential cerebral autoradiography revealed that AngIV facilitated the redistribution of blood flow to ischemic areas within a few minutes. Thus, AngIV may yield its cerebral protective effect against acute cerebral ischemia via an intracerebro-hemodynamic c-Met receptor-mediated NO-dependent mechanism. Given these results a metabolically stable BBB penetrant small molecule that activates the HGF/c-Met system could prove highly efficacious in the treatment of depression.

7. The Development of Angiotensin IV-Based Small Molecules

AngIV-based pharmaceuticals have been suggested as potential anti-dementia therapeutic agents by several investigators [130-133]. In an effort to develop such a drug we synthesized a number of AngIV-based compounds possessing extended half-lives by utilizing amino acid replacement and reduced peptide bonds (CH_2-NH_2) between residues [134,135]. As mentioned earlier this resulted in the development of two potent receptor antagonists, Norleual-AngIV and Divalinal-AngIV [49,74,76,136,137], and one promising agonist, Nle^1-AngIV. About this same time Taisho Pharmaceutical disclosed a series of compounds evaluated in competition binding experiments with [^{125}I]AngIV utilizing guinea pig hippocampal membranes [138,139]. Taisho made use of a styrene moiety to replace three amino acids of AngIV (HPF), and further reduced the amide bond between Y and I.

Although a number of AngIV-based analogs exhibited favorable behavioral results as evaluated using animal models, two critical physiochemical properties continued to hinder drug development. These included: 1) a lack of metabolic stability resulting in short circulating half-lives (eg. Nle^1-AngIV = 1.42 min., [81]; and 2) an inability to penetrate the BBB. This latter limitation of AngIV-related peptides results from considerations of molecular size, overall hydrophobicity, and hydrogen-bonding potential as reflected by the size of the encompassing hydration sphere. Such limitations prompted efforts to design and synthesize new AngIV-based small molecules with these desirable properties.

Members of our laboratory determined that the Nle^1-AngIV agonist effects derived from its N-terminal region given that fragments as small as tetra- and tripeptides retained the ability to overcome scopolamine-induced amnesia [89]. Further, Nle^1-AngIV, as well as these shorter fragments, augmented hippocampal synaptic connectivity via the formation of new synapses [89]. Functionality of these synapses was inferred from analog-induced spinogenesis and the colocalization of synaptic markers in newly formed dendritic spines which were coupled with enhanced miniature excitatory postsynaptic currents. These results encouraged the possibility that a clinically useful small molecule could be designed possessing oral efficacy, increased metabolic stability with an extended half-life, and BBB penetrability. Subsequent design and synthesis efforts

yielded a small molecule with increased hydrophobicity, decreased hydrogen bonding potential, and significantly increased metabolic stability: N-hexanoic-Tyr-Ile-(6) amino hexanoic amide (Dihexa; Fig. 3). This compound induces spinogenesis/synaptogenesis at picomolar concentrations [89] and penetrates the BBB intact and/or as an active metabolite [81].

We reported that Dihexa binds with high affinity to HGF and induces c-Met phosphorylation in the presence of subthreshold levels of HGF [140]. Dihexa also stimulated Hip spinogenesis and synaptogenesis equivalent with HGF. Treatment with the HGF antagonist Hinge (KDYIRN), as well as a short hairpin RNA directed at c-Met, significantly inhibited these actions. Further, Dihexa or an active metabolite penetrated the BBB in sufficient quantity to facilitate memory consolidation and retrieval in the scopolamine-induced amnesic rat model of Alzheimer's disease as well as in aged rats employing the Morris water maze task of spatial memory [81]. These findings have recently been extended to show that by day 8 of testing those animals given Hinge by intracerebroventricular (icv) injection and saline (by gavage) performed equivalently to the control group (icv aCSF followed by saline) [140]. Members of both groups located the hidden platform significantly faster than those animals given scopolamine (icv) followed by saline. Animals treated with scopolamine plus Dihexa (by gavage) performed equivalently with members of the group given aCSF and Dihexa. Finally, those animals given scopolamine and Hinge (icv) followed by Dihexa revealed much slower latencies to find the platform. Taken together these results indicate that Dihexa is capable of reversing the cognitive deficits induced by scopolamine; while the co-application of Hinge and scopolamine blocked the ability of Dihexa to rescue spatial memory. The application of Hinge alone did not interfere with basal performance. This finding is consistent with earlier results utilizing the Morris water maze task showing that icv treatment with AngIV, and AngIV analogs, was ineffective at facilitating learning and memory in normal functioning animals [141]. Thus, it appears that the brain HGF/c-Met system is designed to respond to injury as seen in stroke and neurodegenerative diseases by facilitating synaptic plasticity and neurogenesis. This hypothesis is further supported by transient elevations in CNS HGF levels measured in several degenerative diseases including amyotrophic lateral sclerosis, multiple sclerosis, Parkinson's disease and spinal cord injury [142-145].

8. Conclusion

Major depression is a psychological disorder seen in all age groups. New treatment strategies are needed to address the neuropathology caused by this disease given that upwards of 50% of patients respond poorly to presently available medications. Activation of the HGF/c-Met receptor system may offer neuroprotection to neurotransmitter pathways and promote synaptogenesis and neurogenesis in the Hip and PFC, two structures that show volume reductions with depression. However, the use of HGF has at least two problems: 1) HGF is a large heterodimeric protein that is very expensive to synthesize; and 2) As a large protein HGF does not penetrate the BBB and thus cannot reach brain locations where neurodegeneration is occurring. We have discovered that the small peptide AngIV, and its analogs, facilitate HGF dimerization which is a prerequisite to binding and activation of the c-Met receptor [75,76]. HGF is intimately involved in cell survival, proliferation, migration, and differentiation [55-57], and blunts neurodegenerative influences [62]. However, the AngIV analog Nle^1-AngIV does not readily pass the BBB. Thus, an AngIV-based small molecule, Dihexa was developed that possesses sufficient metabolic stability coupled with BBB penetrability. Dihexa acts via the HGF/c-Met receptor system to facilitate synaptic connectivity and plasticity. The availability of a small molecule HGF mimetic represents a significant advantage over the use of large HGF analogs to accomplish the treatment goal of preventing stress/depression-induced neurodegeneration. It remains to be seen whether treatment of patients with major depression is possible and efficacious using Dihexa.

Conflict of Interests Disclosure

Drs. Wright and Harding are the co-founders of M3 Biotechnology, Inc. and hold stock in this company which is involved in the development of drugs to treat depression, Alzheimer's and Parkinson's diseases. No funds from this company were used to conduct the animal research presented in this manuscript or in the preparation of this review article.

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