Ther Adv Cardiovasc Dis
2015, Vol. 9(4) 209–216
DOI: 10.1177/1753944715599875

Angiotensin-(1–7): beyond its central effects on blood pressure

Mariela M. Gironacci

***Abstract*:** Angiotensin (Ang) (1–7) is the main component of the depressor and protective arm of the renin-angiotensin system. Ang-(1–7) induces vasodilation, natriuresis and diuresis, cardioprotection, inhibits angiogenesis and cell growth and opposes the pressor, proliferative, profibrotic, and prothrombotic actions mediated by Ang II. Centrally, Ang-(1–7) induces changes in mean arterial pressure and this effect may be linked with its inhibitory neuromodulatory action on norepinephrine neurotransmission. The present review is focused on the role of Ang-(1–7) as a protective agent in the brain.

Keywords: angiogenesis, angiotensin-(1–7), Mas receptor, norepinephrine, stroke

Correspondence to:
Mariela M. Gironacci, PhD
Departamento de Química Biológica, IQUIFIB-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 Ciudad Autónoma de Buenos Aires, Argentina
mariela@qb.ffyb.uba.ar

Introduction

The renin–angiotensin system (RAS) is composed of two arms. The pressor arm is represented mainly by angiotensin (Ang) II, the angiotensin-converting enzyme (ACE) and the Ang type 1 receptor (AT1R), while the depressor or protective arm is mainly represented by Ang-(1–7), Ang type 2 receptor (AT2R), the Mas receptor [through which Ang-(1–7) exerts its action] and ACE2. The Mas receptor, which was first described to be specific for Ang-(1–7) by Santos and colleagues in 2003 [Santos *et al.* 2003], belongs to the G protein-coupled receptor family, but is distinct from AT1R and AT2R. Several reports showed the lack of activation of conventional G protein signaling pathways upon stimulation with Ang-(1–7). For example, in Mas expressing cells the intracellular levels of the classical G protein-induced second messenger molecules such as calcium or inositol 1,4,5-trisphosphate were not altered upon Ang-(1–7) treatment [Tirupula *et al.* 2014]. Conversely, Mas activation leads to prostaglandin and nitric oxide (NO) generation as well as phosphoinositide 3 kinase (PI3K)/Akt and mitogen-activated protein kinase kinase (MEK) 1/2/extracellular-signal-regulated kinase (ERK) 1/2 pathways stimulation [Gironacci *et al.* 2014].

Ang-(1–7) is an endogenous counter-regulator of Ang II because it produces vasodilation, natriuresis and diuresis, and cardioprotection, inhibits angiogenesis and cell growth and opposes the pressor, proliferative, profibrotic and prothrombotic actions mediated by Ang II [Santos *et al.* 2013; Santos, 2014; Varagic *et al.* 2014].

All the components of the RAS are present in the brain [Karamyan and Speth, 2007]. Ang-(1–7) is formed from Ang I through cleavage at the Pro^{7}–Phe^{8} peptide linkage by several endopeptidases, such as prolyl endopeptidase, thimet oligopeptidase or neutral endopeptidase (neprylisin) [Karamyan and Speth, 2007; Rice *et al.* 2004]. Alternatively, Ang-(1–7) is also formed from Ang II being processed by endopeptidases or carboxypeptidases which remove the carboxyl terminal phenylalanine, with ACE2 the primary enzyme (Figure 1) [Rice *et al.* 2004; Vickers *et al.* 2002]. The catalytic efficiency of ACE2 to generate Ang-(1–7) from Ang II is almost 500-fold greater than that shown for conversion of Ang I to Ang-(1–9) and 10- or 600-fold higher than that described for two other Ang-(1–7) forming enzymes, prolyl endopeptidase and prolyl carboxypeptidase, respectively [Vickers *et al.* 2002]. In fact, the protective effects of cerebral ACE2 on neurogenic hypertension and sympathetic activity are closely linked to the Ang-(1–7)/Mas receptor pathway [reviewed by Gironacci *et al.* 2014], showing the contribution of central ACE2 to Ang-(1–7) generation.

Ang-(1–7) in stroke

Stroke is one of the leading causes of death and impaired quality of life as a result of neurological

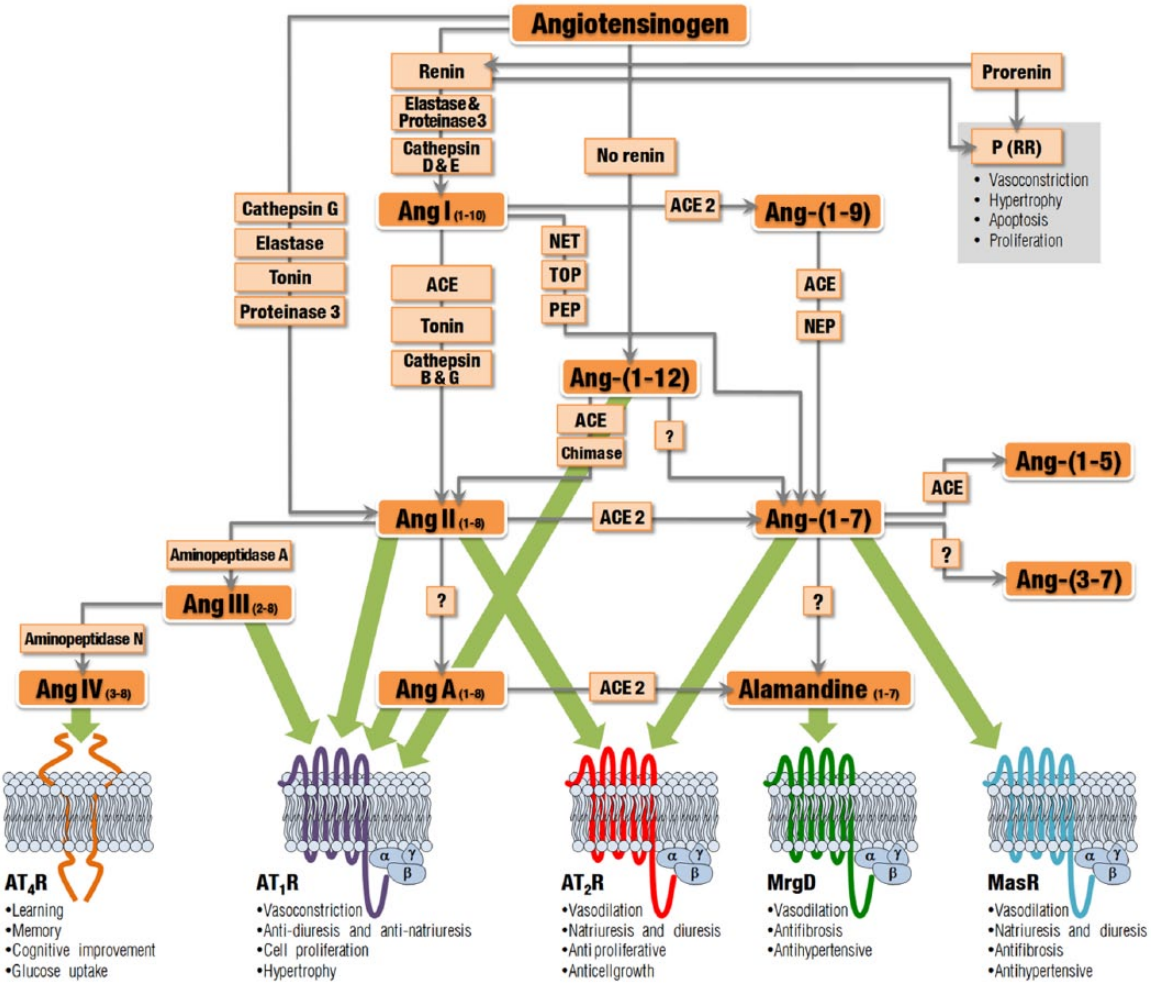

Figure 1. Brain renin–angiotensin system.
ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; AT_1 R, angiotensin type 1 receptor; AT_2 R, angiotensin type 2 receptor; AT_4 R, angiotensin type 4 receptor; Mas R, Mas receptor; MrgD, Mas related G-protein coupled receptors; NEP, neutral endopeptidase (neprilysin); PEP, prolyl endopeptidase; (P)RR, prorenin receptor; TOP, thimet oligopeptidase.

deficit. Stroke occurs when blood flow to the brain is interrupted, creating a central area of cell death (the core) surrounded by compromised tissue (the penumbra). Although the core of the damaged tissue is often beyond repair, the penumbra can recover function if blood flow is restored in a timely manner [Willis *et al.* 2011]. The pressor arm of the brain RAS is involved in ischemic brain damage after stroke. Numerous studies have demonstrated that AT1R blocker treatment can reduce infarct size and improve behavioral recovery in stroke models in normal rats, spontaneously hypertensive rats (SHR) and in atherosclerotic apolipoprotein E deficient mice [Willis *et al.* 2011]. Conversely, central administration of Ang-(1–7) reduces brain damage and improves neurological outcome in ischemic stroke [Mecca *et al.* 2011].

Intracerebroventricular (i.c.v.) infusion of Ang-(1–7) prior to and during ischemic stroke elicited by endothelin1-induced middle cerebral artery occlusion (MCAO) produced a significant reduction in the resulting intracerebral (cortical and striatal) infarct. This decrease in intracerebral infarct size was associated with increased neuron survival in the cortex and striatum, as well as decreases in the behavioral deficits caused by MCAO [Mecca *et al.* 2011]. A similar protective effect of Ang-(1–7) has been documented in a rat model of permanent MCAO [Jiang *et al.* 2012, 2014]. Supporting these findings is the fact that i.c.v. administration of the ACE2 activator, diminazene aceturate, under similar conditions as those used for Ang-(1–7) effectively decreased the intracerebral infarct and behavioral deficits

resulting from endothelin-1-induced MCAO [Mecca *et al.* 2011]. These protective actions of Ang-(1–7) and diminazene aceturate were abolished by co-administration of the receptor Mas blocker, suggesting that is a Mas receptor mediated effect and supporting the involvement of Ang-(1–7) in the protective effects of the ACE2 activator [Mecca *et al.* 2011; Sumners *et al.* 2013]. In another model of stroke as in stroke-prone SHR, which is an established animal model of hypertension induced hemorrhagic stroke, central administration of Ang-(1–7) increases lifespan and improves the neurological status of these rats as well as reducing microglial numbers in the striatum, implying attenuation of cerebral inflammation [Regenhardt *et al.* 2014].

The beneficial actions of Ang-(1–7) in ischemic stroke are due to anti-inflammatory mechanisms. Thus, reductions in inducible nitric oxide synthase (NOS) gene expression, in the pro-inflammatory interleukin (IL) 1b and IL-6, and in microglial activation were elicited by Ang-(1–7) [Regenhardt *et al.* 2013; Sumners *et al.* 2013]. In addition, Ang-(1–7) decreased the levels of oxidative stress and suppressed nuclear factor-κB (NF-κB) activity, a transcriptional regulator involved in inflammation, which was accompanied by a reduction of pro-inflammatory cytokines and cyclooxynease-2 (COX-2) in the peri-infarct regions [Jiang *et al.* 2012]. Despite the fact that AT2Rs have been shown to be recognized by Ang-(1–7) [Bosnyak *et al.* 2011] and that a neuroprotective action has been reported for AT2Rs [Mogi and Horiuchi, 2013], the cerebroprotective effect of Ang-(1–7) was independent of AT2R activation. Ang-(1–7) beneficial effects on ischemic stroke were reversed by the Mas receptor antagonist but not by the AT2R antagonist, suggesting the involvement of the Mas receptor. Conversely, infusion of the Mas receptor antagonist alone increased oxidative stress levels and enhanced NF-κB activity, which was accompanied by an upregulation of pro-inflammatory cytokines and COX-2 [Jiang *et al.* 2012].

Neuronal overexpression of ACE2 protects the brain from ischemia-induced damage [Chen *et al.* 2014]. Overexpression of ACE2 in the neurons of human renin and angiotensinogen double transgenic mice, a model with central Ang II overproduction, displayed less MCAO-induced infarct volume and increased cerebral blood flow, neurological function and cerebral microvascular density in the peri-infarct area [Chen *et al.* 2014]. Blockade of the Mas receptor pathway in the brain partially abolished the beneficial effects of ACE2 overexpression. Furthermore, Ang (1-7)/Ang II ratio, angiogenic factors, endothelial NOS expression and NO production were increased, whereas NADPH oxidase subunits and reactive oxygen species were decreased in the brain of these transgenic mice [Chen *et al.* 2014]. These effects were independent of mean arterial pressure changes. Altogether, these results demonstrate that neuronal ACE2 protects brain from ischemic injury by changing the Ang-(1–7)/Ang II ratio [Chen *et al.* 2014]. The protective effect of neuronal ACE2 overexpression on ischemia was greater in older animals [Zheng *et al.* 2014b]. This was evidenced by lower neurological deficit scores and smaller stroke volumes in eight-month old transgenic mice compared with three-month ones [Zheng *et al.* 2014b]. In addition, brain tissue from these human renin and angiotensinogen transgenic mice with overproduction of ACE2 in neurons displayed less swelling and cell death in response to oxygen and glucose deprivation. This effect was blocked by the Mas receptor antagonist, suggesting that Ang-(1–7) production which results from ACE2 overexpression may exert this protective effect [Zheng *et al.* 2014a]. Thus, these data provide evidence that activation of ACE2/Ang-(1–7)/Mas pathway can exert a direct neuroprotective action by alleviating ischemia induced cell swelling and cell death [Zheng *et al.* 2014a].

Supporting the beneficial effects of the depressor arm of the RAS, it has been shown that the expression of the ACE2/Ang-(1–7)/Mas receptor axis is upregulated after acute cerebral ischemic stroke in rats [Lu *et al.* 2013]. Cerebral ischemic injury resulted in a significant increase of cerebral and circulating Ang-(1–7) at 6–48 hours following focal ischemic stroke compared with the control. Both Mas and ACE2 expression in the ischemic tissues and blood serum were markedly enhanced as a result of the acute ischemic insult. The Mas immunopositive neurons showed stronger expression in the ischemic cortex [Lu *et al.* 2013].

Ang-(1–7) and angiogenesis

Angiogenesis is a target for recovery after an ischemic stroke. Human studies have demonstrated that increased angiogenesis in the penumbra region is correlated with increased survival in stroke patients [Willis *et al.*2011]. The correlation between angiogenesis and improved functional outcome after ischemic stroke remains, and is

seen in both animal models and in human stroke patients [Ergul *et al.* 2012]. The growth factors expressed may promote survival of the endothelial, glial and neuronal cell types in the penumbral area and the neovascularization may act to remove damaged tissue [Ergul *et al.* 2012]. Ang-(1–7) promotes brain angiogenesis. Infusion of Ang-(1–7) for 4 weeks promotes endothelial cell proliferation and increases brain capillary density in rats with permanent MCAO, which was accompanied by endothelial nitric oxide synthase (eNOS) activation and upregulation of nitric oxide (NO). Furthermore, Ang-(1–7)-induced brain angiogenesis attenuates the reduction of regional cerebral blood flow during subsequent ischemia and leads to the improvement in stroke outcome [Jiang *et al.* 2014]. Vascular endothelial growth factor (VEGF) is among the growth factors implicated in the recovery process after ischemic stroke [Ergul *et al.* 2012; Willis *et al.* 2011]. The level of VEGF, a main downstream effector of eNOS, was also increased in brain after Ang-(1–7) infusion. All these effects could be completely abolished by the Mas receptor blocker, implying that eNOS was a downstream effector of Ang-(1–7)/Mas signaling [Jiang *et al.* 2014].

Despite the fact that Ang-(1–7) and Mas receptor have been demonstrated to be involved in the generation of new blood vessels, little is known about their role in neuron generation. Mas expression was identified in dividing cells and in doublecortin-positive young neurons within the dentate gyrus of mice, one of the brain areas capable of adult neurogenesis [Freund *et al.* 2014]. Doublecortin is expressed by late mitotic active and early postmitotic young neurons, and may account for increased neurogenesis or maturation rate of this neuronal population. Lack of Mas, however, did not significantly affect cell proliferation and significantly increased the population of doublecortin-positive cells, indicating that Mas has a specific role for this neuronal population [Freund *et al.* 2014]. These results suggest that blockade of Mas might be beneficial in stimulating neurogenesis in adults.

Ang-(1–7) and cognitive function

Maintenance of brain function depends on a constant blood supply. Deficits in cerebral blood flow are linked to cognitive decline and they have detrimental effects on the outcome of ischemia [Pires *et al.* 2013]. Hypertension causes alterations in cerebral artery structure and function that can impair blood flow, particularly during an ischemic insult or during periods of low arterial pressure [Pires *et al.* 2013]. Chronic cerebral hypoperfusion is associated with cognitive decline in aging, vascular dementia and Alzheimer's disease. Indeed, a role for the brain RAS in Alzheimer's disease has been reported [Wright *et al.* 2013]. Ang-(1–7) was found to protect against cognitive dysfunction [Xie *et al.* 2014]. Ang-(1–7) significantly alleviated chronic cerebral hypoperfusion-induced cognitive deficits in rats subjected to permanent bilateral occlusion of the common carotid arteries, a model of chronic cerebral hypoperfusion. This neuroprotective effect was associated with increased NO generation, attenuated neuronal loss and suppressed astrocyte proliferation in the hippocampus [Xie *et al.* 2014]. Recently, it has been shown that Ang-(1–7) levels decreased in the brain of an animal model of Alzheimer's disease during disease progression [Jiang *et al.* 2015]. In addition, an inverse correlation was found between Ang-(1–7) and tau hyperphosphorylation in brain, suggesting that Ang-(1–7) might be involved in the etiology and pathogenesis of Alzheimer's disease, possibly *via* modulation of tau hyperphosphorylation [Jiang *et al.* 2015].

Ang-(1–7) protects the blood–brain barrier and neurons

Hypertension causes blood–brain barrier breakdown by mechanisms involving inflammation, oxidative stress and vasoactive circulating molecules. This exposes neurons to cytotoxic molecules, leading to neuronal loss, cognitive decline and impaired recovery from ischemia [Pires *et al.* 2013]. Recently, it has been shown that Ang-(1–7) exerts a protective role in blood–brain barrier damage. In cerebral ischemia reperfusion injury-induced and hypoxia-induced blood–brain barrier damage, Ang-(1–7) promotes the expression of zonula occludens-1 and claudin-5, which are proteins associated with tight junction in cerebral endothelial cells of the blood brain barrier [Wu *et al.* 2015].

In an attempt to elucidate the cellular target for the protective effect elicited by Ang-(1–7) in the brain, we investigated the different cellular type protected by Ang-(1–7) by transmission electron microscopy in the model of brain damage induced by Shiga toxin 2 (Stx2) producing enterohemorrhagic *Escherichia coli*. Stx2 induced neurodegeneration and axon demyelination. Ang-(1–7) prevented neuronal damage and hampered the Stx2-induced demyelination [Gironacci *et al.* 2013]. These effects were mediated by the Mas receptor (unpublished results).

Central Ang-(1–7) improves peripheral cardiovascular responses

Ang-(1–7) not only acts as a cerebroprotective when it is given in the brain. It is well known that Ang-(1–7) acting in different nucleus of the brain induces changes in arterial blood pressure (reviewed by Gironacci *et al.* 2013, 2014). For instance, a reduction in blood pressure occurred after i.c.v. Ang-(1–7) was observed in the nucleus of the nucleus tractus solitarii, caudal ventrolateral medulla, paraventricular nucleus or anterior hypothalamic area, while an increase in blood pressure was observed in the rostral ventrolateral medulla [Gironacci *et al.* 2013]. Recently, it has been shown that chronic increase in Ang-(1–7) levels in the brain improves both cardiovascular and metabolic parameters in an experimental model of metabolic syndrome [Guimaraes *et al.* 2014]. Chronic i.c.v. infusion of Ang-(1–7) attenuated the development of hypertension and improved the baroreflex control of heart rate and the glucose metabolism in fructose fed rats [Guimaraes *et al.* 2014].

Ang-(1–7) can act in the amygdala to attenuate the cardiovascular response to acute emotional stress. Injection of Ang-(1–7) into the basolateral amygdala blocked the tachycardia and the pressor response produced by air jet stress [Oscar *et al.* 2015]. These effects were completely reversed by the Mas receptor antagonist, indicating that Ang-(1–7) through the Mas receptors modulates the cardiovascular response evoked by emotional stress [Oscar *et al.* 2015]. Similar attenuating stress-induced tachycardia response were reported when Ang-(1–7) or the ACE2 activator compound, 1-[[2-(dimethylamino)ethyl]amino]-4-(hydroxymethyl)-7-[[(4-methylphenyl)sulfonyl]oxy]-9H-xanthen-9-one (XNT) were injected i.c.v. [Martins Lima *et al.* 2013]. XNT was able to evoke wide cardiovascular responses [Martins Lima *et al.* 2013]. XNT would achieve its effects not by forming only Ang-(1–7), but also by degrading Ang II. Thus, Ang-(1–7) can act in central circuits modulating anxiety and emotional stress responses.

Ang-(1–7) and norepinephrine

Ang-(1–7) central effects on blood pressure regulation and cardiovascular responses may be associated with changes in neurotransmitter synaptic levels. Sympathetic nervous activity in both central and peripheral nervous systems may play a major role in the regulation of blood pressure, and that hypertension is accompanied characteristically by increased sympathetic nervous activity in both humans and animal models [Tsuda, 2012]. The brain RAS may actively participate in the modulation of neurotransmitter release and influence the central sympathetic outflow to the periphery [Tsuda, 2012]. Ang II is well known to facilitate norepinephrine (NE) neurotransmission [Tsuda, 2012; Veersingham and Raizada, 2003]. In contrast to Ang II, Ang-(1–7) elicits a sympato-inhibitory action [Gironacci *et al.* 2013]. In neurons from the hypothalamus and brainstem of normotensive and SHR, Ang-(1–7) leads to a decrease in NE levels in the synaptic cleft. This effect results from a decrease in neurotransmitter release and synthesis and an increase in its neuronal uptake (Figure 2). Thus, Ang-(1–7) through Mas and AT2Rs elicits a reduction in NE release in hypothalami from normotensive and hypertensive rats in a bradykinin/NO-dependent manner through the cyclic guanosine monophosphate (cGMP)/protein kinase G (PKG) pathway which, in turn, maintains low NE outflow [Gironacci *et al.* 2000, 2004].

Ang-(1–7) not only induces a decrease in neurotransmitter release but also in its synthesis. The peptide, through an AT2R-mediated mechanism, downregulates tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of catecholamines, reducing in consequence NE biosynthesis. Ang-(1–7) downregulates TH expression by increasing its degradation through stimulation of the ubiquitin–proteasome system, which is the major pathway for intracellular protein degradation in eukaryotic cells [Lopez Verrilli *et al.* 2009].

Synaptic neurotransmission requires the precise control of the duration and the magnitude of neurotransmitter action at specific molecular targets. Uptake of monoamine neurotransmitters into presynaptic terminals through the transporters is the main mechanism for monoaminergic neurotransmission ending. NE transporter (NET) regulates the clearance of NE from the synaptic cleft. Changes in the activity of NET should have a significant impact on the concentration and duration of NE present in the synaptic cleft, and thus NET is essential for a fine-tuned control of sympathetic activity [Bönisch and Brüss, 2006; Kvetnansky *et al.* 2009]. Ang-(1–7) does not evoke an acute effect on neuronal NE uptake. Conversely, Ang-(1–7) causes a long-term stimulatory effect on NE neuronal uptake by increasing NET transcription and expression. The Ang-(1–7)-stimulated NET expression is coupled to Mas receptor activation acting through a

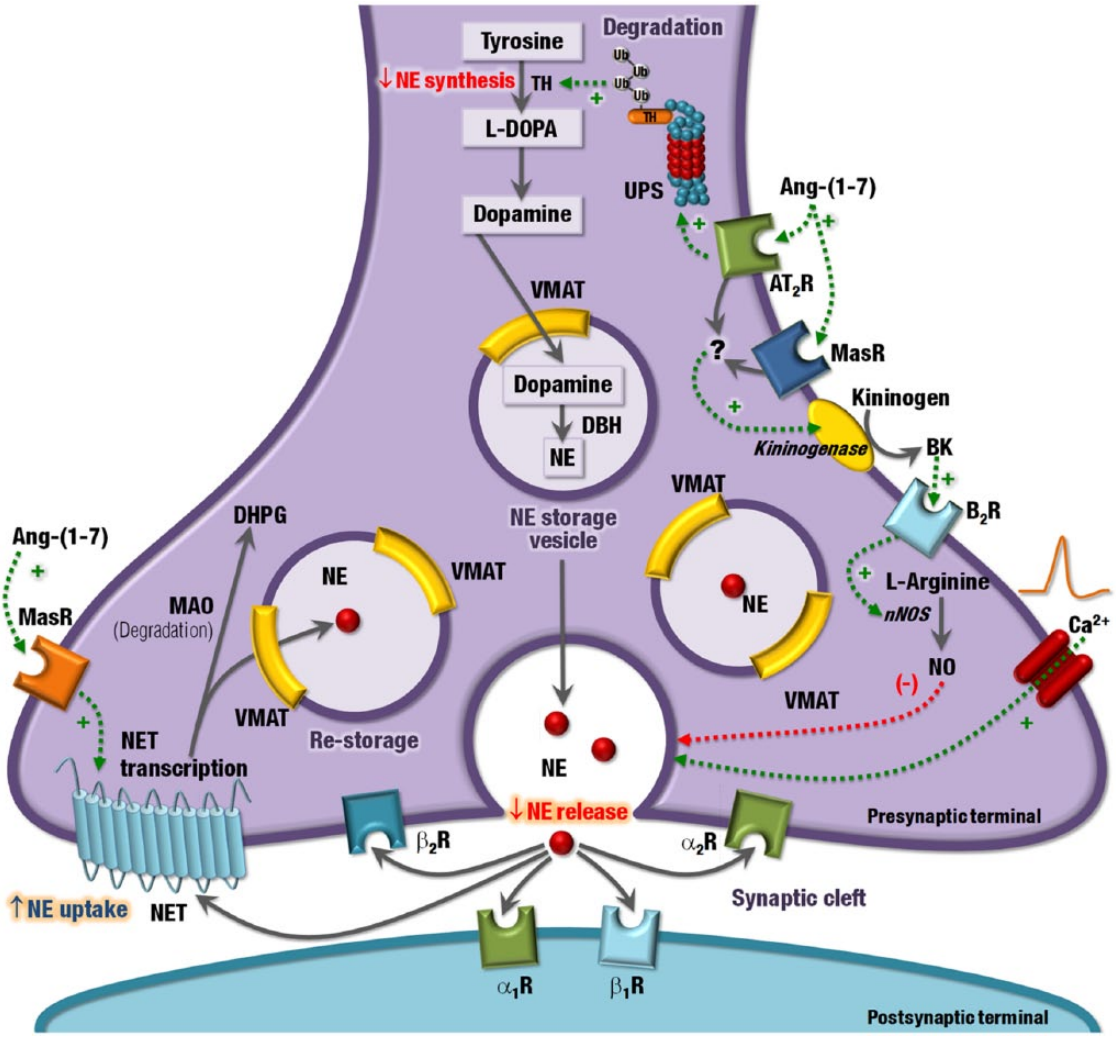

Figure 2. Schematic representation of a sympathetic neuron and the targets of Ang-(1–7) action which results in norepinephrine (NE) levels changes in the synaptic cleft.
Ang, angiotensin; AT_2R, angiotensin type 2 receptor; BK, bradykinin; B_2R, bradykinin B_2 receptor; DBH, dopamine β-hydroxylase; DHPG, dihydroxyphenylglycine; L-DOPA, l-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; MasR, Mas receptor; NET, norepinephrine transporter; NO, nitric oxide; nNOS, neuronal nitric oxide synthase; TH, tyrosine hydroxylase; UPS, ubiquitin–proteasome system; VMAT, vesicular monoamine transporter; α_1R, α_1-adrenergic receptor; α_2R, α_2-adrenergic receptor; β_1R, β_1-adrenergic receptor; β_2R, β_2-adrenergic receptor; Ub, ubiquitin.

PI3-kinase/Akt and MEK 1/2-ERK1/2-dependent pathway [Lopez Verrilli *et al.* 2012].

Altogether, these results showed that Ang-(1–7) elicits a negative neuromodulatory role on NE neurotransmission, thus contributing to the modulation of NE homeostasis and maintaining appropriate synaptic NE levels during hypertensive conditions.

Conclusion

To date it is clear the role of Ang-(1–7) as a cerebroprotective agent. Therapies that induce an increase in centrally Ang-(1–7) levels may be considered possible approaches as neuroprotectives. For instance, ACE inhibitors lead to an increase in Ang-(1–7) levels [Ferrario *et al.* 2005] and they are widely used in stroke treatment. The ACE inhibitor captopril given subcutaneously has been shown to be neuroprotective in animal models of parkinsonism [Sonsalla *et al.* 2013]. Inhibition of ACE activity not only increases the generation of Ang-(1–7) from Ang I, but also reduces its degradation. Another possible therapy may be the systemic administration of an ACE2 activator, which favors Ang II degradation with the subsequent increase in Ang-(1–7) levels.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of interest statement

The author declares no conflicts of interest in preparing this article.

References

Bönisch, H. and Brüss, M. (2006) The norepinephrine transporter in physiology and disease. *Handb Exp Pharmacol* 175: 485–524.

Bosnyak, S., Jones, E., Christopoulos, A., Aguilar, M., Thomas, W. and Widdop, R. (2011) Relative affinity of angiotensin peptides and novel ligands at AT1 and AT2 receptors. *Clin Sci* 121: 297–303.

Chen, J., Zhao, Y., Chen, S., Wang, J., Xiao, X., Ma, X. *et al.* (2014) Neuronal over-expression of ACE2 protects brain from ischemia-induced damage. *Neuropharmacology* 79: 550–558.

Ergul, A., Alhusban, A. and Fagan, S. (2012) Angiogenesis: a harmonized target for recovery after stroke. *Stroke* 43: 2270–2274.

Ferrario, C., Trask, A. and Jessup, J. (2005) Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1–7) in regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol* 289: H2281–H2290.

Freund, M., Walther, T. and von Bohlen Und Halbach, O. (2014) Effects of the angiotensin-(1–7) receptor Mas on cell proliferation and on the population of doublecortin positive cells within the dentate gyrus and the piriform cortex. *Eur Neuropsychopharmacol* 24: 302–308.

Gironacci, M., Cerniello, F., Longo Carbajosa, N., Goldstein, J. and Cerrato, B. (2014) Protective axis of the renin–angiotensin system in the brain. *Clin Sci* 127: 295–306.

Gironacci, M., Longo Carbajosa, N., Goldstein, J. and Cerrato, B. (2013) Neuromodulatory role of angiotensin-(1–7) in the central nervous system. *Clin Sci* 125: 57–65.

Gironacci, M., Valera, M., Yujnovsky, I. and Peña, C. (2004) Angiotensin-(1–7) inhibitory mechanism of norepinephrine release in hypertensive rats. *Hypertension* 44: 783–787.

Gironacci, M., Vatta, M., Rodriguez-Fermepin, M., Fernandez, B. and Peña, C. (2000) Angiotensin-(1–7) reduces norepinephrine release through a nitric oxide mechanism in rat hypothalamus. *Hypertension* 35: 1248–1252.

Guimaraes, P., Oliveira, M., Braga, J., Nadu, A., Schreihofer, A., Santos, R. *et al.* (2014) Increasing angiotensin-(1–7) levels in the brain attenuates metabolic syndrome-related risks in fructose-fed rats. *Hypertension* 63: 1078–1085.

Jiang, T., Gao, L., Guo, J., Lu, J., Wang, Y. and Zhang, Y. (2012) Suppressing inflammation by inhibiting the NF-kappaB pathway contributes to the neuroprotective effect of angiotensin-(1–7) in rats with permanent cerebral ischaemia. *Br J Pharmacol* 167: 1520–1532.

Jiang, T., Yu, J., Zhu, X., Zhang, Q., Tan, M., Cao, L. *et al.* (2014) Angiotensin-(1–7) induces cerebral ischaemic tolerance by promoting brain angiogenesis in a Mas/eNOS-dependent pathway. *Br J Pharmacol* 171: 4222–4232.

Jiang, T., Zhang, Y., Zhou, J., Zhu, X., Tian, Y. and Zhao, H. *et al.* (2015) Angiotensin-(1–7) is reduced and inversely correlates with tau hyperphosphorylation in animal models of Alzheimer's disease. *Mol Neurobiol.* 5 June 2015. [Epub ahead of print]

Karamyan, V. and Speth, R. (2007) Enzymatic pathways of the brain renin-angiotensin system: unsolved problems and continuing challenges. *Regul Pept* 143: 15–27.

Kvetnansky, R., Sabban, E. and Palkovits, M. (2009) Catecholaminergic systems in stress: structural and molecular genetic approaches. *Physiol Rev* 89: 535–606.

Lopez Verrilli, M., Pirola, C., Pascual, M., Dominici, F., Turyn, D. and Gironacci, M. (2009) Angiotensin-(1–7) through AT receptors mediates tyrosine hydroxylase degradation *via* the ubiquitin-proteasome pathway. *J Neurochem* 109: 326–335.

Lopez Verrilli, M., Rodriguez Fermepín, M., Longo Carbajosa, N., Landa, S., Cerrato, B., Garcia, S. *et al.* (2012) Angiotensin-(1–7) through Mas receptor up-regulates neuronal norepinephrine transporter *via* Akt and Erk1/2-dependent pathways. *J Neurochem* 120: 46–55.

Lu, J., Jiang, T., Wu, L., Gao, L., Wang, Y., Zhou, F. *et al.* (2013) The expression of angiotensin-converting enzyme 2-angiotensin-(1–7)-Mas receptor axis are upregulated after acute cerebral ischemic stroke in rats. *Neuropeptides* 47: 289–295.

Martins Lima, A., Xavier, C., Ferreira, A., Raizada, M., Wallukat, G., Velloso, E. *et al.* (2013) Activation of angiotensin-converting enzyme 2/angiotensin-(1–7)/Mas axis attenuates the cardiac reactivity to acute emotional stress. *Am J Physiol Heart Circ Physiol* 305: H1057–H1067.

Mecca, A., Regenhardt, R., O'Connor, T., Joseph, J., Raizada, M., Katovich, M. *et al.* (2011) Cerebroprotection by angiotensin-(1–7) in endothelin-1-induced ischaemic stroke. *Exp Physiol* 96: 1084–1096.

Mogi, M. and Horiuchi, M. (2013) Effect of angiotensin II type 2 receptor on stroke, cognitive impairment and neurodegenerative diseases. *Geriatr Gerontol Int* 13: 13–18.

Oscar, C., Müller-Ribeiro, F., de Castro, L., Martins Lima, A., Campagnole-Santos, M., Santos, R. *et al.* (2015) Angiotensin-(1–7) in the basolateral amygdala attenuates the cardiovascular response evoked by acute emotional stress. *Brain Res* 1594: 183–189

Pires, P., Dams Ramos, C., Matin, N. and Dorrance, A. (2013) The effects of hypertension on the cerebral circulation. *Am J Physiol Heart Circ Physiol* 304: H1598–H1614.

Regenhardt, R., Desland, F., Mecca, A., Pioquinto, D., Afzal, A., Mocco, J. *et al.* (2013) Anti-inflammatory effects of angiotensin-(1–7) in ischemic stroke. *Neuropharmacology* 71: 154–163.

Regenhardt, R., Mecca, A., Desland, F., Ritucci-Chinni, P., Ludin, J., Greenstein, D. *et al.* (2014) Centrally administered angiotensin-(1–7) increases the survival of stroke-prone spontaneously hypertensive rats. *Exp Physiol* 99: 442–453.

Rice, G., Thomas, D., Grant, P., Turner, A. and Hooper, N. (2004) Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem J* 383: 45–51.

Santos, R. (2014) Angiotensin-(1–7). *Hypertension* 63: 1138–1147.

Santos, R., Ferreira, A., Verano-Braga, T. and Bader, M. (2013) Angiotensin-converting enzyme 2, angiotensin-(1–7) and Mas: new players of the renin-angiotensin system. *J Endocrinol* 216: R1–R17.

Santos, R., Simoes e Silva, A., Maric, C., Silva, D., Machado, R., de Buhr, I. *et al.* (2003) Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc Natl Acad Sci U S A* 100: 8258–8263.

Sonsalla, P., Coleman, C., Wong, L., Harris, S., Richardson, J., Gadad, B. *et al.* (2013) The angiotensin converting enzyme inhibitor captopril protects nigrostriatal dopamine neurons in animal models of parkinsonism. *Exp Neurol* 250: 376–383.

Sumners, C., Horiuchi, M., Widdop, R., McCarthy, C., Unger, T. and Steckelings, U. (2013) Protective arms of the renin-angiotensin-system in neurological disease. *Clin Exp Pharmacol Physiol* 40: 580–588.

Tirupula, K., Desnoyer, R., Speth, R. and Karnik, S. (2014) Atypical signaling and functional desensitization response of MAS receptor to peptide ligands. *PLoS One* 9: e103520.

Tsuda, K. (2012) Renin-Angiotensin system and sympathetic neurotransmitter release in the central nervous system of hypertension. *Int J Hypertens* 2012: 474870

Varagic, J., Ahmad, S., Nagata, S. and Ferrario, C. (2014) ACE2: angiotensin II/angiotensin-(1–7) balance in cardiac and renal injury. *Curr Hypertens Rep* 16: 420.

Veersingham, S. and Raizada, M. (2003) Brain renin-angiotensin system in hypertension. *Brit J Pharmacol* 139: 191–202.

Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J. *et al.* (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J Biol Chem* 277: 14838–14843.

Willis, L., El-Remessy, A., Somanath, P., Deremer, D. and Fagan, S. (2011) Angiotensin receptor blockers and angiogenesis: clinical and experimental evidence. *Clin Sci* 120: 307–319.

Wu, J., Zhao, D., Wu, S. and Wang, D. (2015) Ang-(1–7) exerts protective role in blood-brain barrier damage by the balance of TIMP-1/MMP-9. *Eur J Pharmacol* 748: 30–36.

Wright, J., Kawas, L. and Harding, J. (2013) A role for the brain RAS in Alzheimer's and Parkinson's diseases. *Front Endocrinol* 4: 158.

Xie, W., Zhu, D., Ji, L., Tian, M., Xu, C. and Shi, J. (2014) Angiotensin-(1–7) improves cognitive function in rats with chronic cerebral hypoperfusion. *Brain Res* 1573: 44–53.

Zheng, J., Li, G., Chen, S., Bihl, J., Buck, J., Zhu, Y. *et al.* (2014a) Activation of the ACE2/Ang-(1–7)/Mas pathway reduces oxygen-glucose deprivation-induced tissue swelling, ROS production, and cell death in mouse brain with angiotensin II overproduction. *Neuroscience* 273: 39–51.

Zheng, J., Li, G., Chen, S., Wang, J., Olson, J., Xia, H. *et al.* (2014b) Angiotensin converting enzyme 2/Ang-(1–7)/Mas axis protects brain from ischemic injury with a tendency of age-dependence. *CNS Neurosci Ther* 20: 452–459.