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Neuromodulatory role of angiotensin-(1–7) in the central nervous system

Mariela M. GIRONACCI*, Nadia A. LONGO CARBAJOSA*, Jorge GOLDSTEIN† and Bruno D. CERRATO*

*Departamento de Química Biológica, Instituto de Química y Fisicoquímica Biológicas-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina
†Laboratorio de Neurofisiopatología, Facultad de Medicina, Universidad de Buenos Aires, Argentina

Abstract

Ang-(1–7) [angiotensin-(1–7)] constitutes an important functional end-product of the RAS (renin–angiotensin system) endogenously formed from AngI (angiotensin I) or AngII (angiotensin II) through the catalytic activity of ACE2 (angiotensin-converting enzyme 2), prolyl carboxypeptidase, neutral endopeptidase or other endopeptidases. Ang-(1–7) lacks the pressor, dipsogenic or stimulatory effect on aldosterone release characteristic of AngII. In contrast, it produces vasodilation, natriuresis and diuresis, and inhibits angiogenesis and cell growth. At the central level, Ang-(1–7) acts at sites involved in the control of cardiovascular function, thus contributing to blood pressure regulation. This action may result from its inhibitory neuromodulatory action on NE [noradrenaline (norepinephrine)] levels at the synaptic cleft, i.e. Ang-(1–7) reduces NE release and synthesis, whereas it causes an increase in NE transporter expression, contributing in this way to central NE neuromodulation. Thus, by selective neurotransmitter release, Ang-(1–7) may contribute to the overall central cardiovascular effects. In the present review, we summarize the central effects of Ang-(1–7) and the mechanism by which the peptide modulates NE levels in the synaptic cleft. We also provide new evidences of its cerebroprotective role.

Key words: angiotensin-(1–7), hypertension, Mas receptor, noradrenaline (norepinephrine), synapse, tyrosine hydroxylase

ANGIOTENSIN-(1–7)

The RAS (renin–angiotensin system) is one of the major systems in the regulation of cardiovascular function and fluid homoeostasis. The classical RAS is an enzymatic cascade by which angiotensinogen is cleaved by renin and then by ACE (angiotensin-converting enzyme) to produce AngII (angiotensin II) and subsequently cleaved to form other angiotensins (Figure 1). A complete RAS exists in the brain and comprises all necessary precursors and enzymes required for formation and metabolism of its components [1,2].

Within the brain, AngII contributes to cardiovascular regulation via its action at various hypothalamic and medullary areas, resulting in elevated blood pressure, augmented drinking behaviour, attenuation of the baroreflex, enhancement of sympathetic outflow and augmented vasopressin release [1–3].

AngII is certainly not the only biologically active peptide of the RAS, since it has been determined that other peptides contribute to or actually oppose the pressor and proliferative actions of AngII [2,4–6]. Among them, Ang-(1–7) [angiotensin-(1–7)] is an endogenous counter-regulator of AngII because it produces vasodilation, natriuresis and diuresis, cardioprotection, inhibits angiogenesis and cell growth and opposes the pressor, proliferative, profibrotic and prothrombotic actions mediated by AngII [6–8].

Ang-(1–7) is formed by a route independent of ACE, the peptidase involved in AngII generation. Ang-(1–7) can be formed from AngI (angiotensin I) through cleavage at the Pro^{7}-Phe^{8} peptide linkage by several endopeptidases, such as prolyl endopeptidase, thimet oligopeptidase or neutral endopeptidase (neprilysin) [7,9]. Alternatively, AngII processing by endo- or carboxypeptidases, which remove the C-terminal phenylalanine, can produce Ang-(1–7), with ACE2 acting as the primary enzyme [9–11] (Figure 1). Since ACE2 degrades the pressor peptide AngII to the antihypertensive component of the RAS Ang-(1–7), ACE2 plays a critical role in regulating the balance between vasoconstrictor and vasodilator effects within the RAS cascade [7].

Abbreviations: ACE, angiotensin-converting enzyme; Ang-(1–7), angiotensin-(1–7); AngII, angiotensin II; AT_1, angiotensin type 1; AT_2, angiotensin type 2; CNS, central nervous system; CVLM, caudal ventrolateral medulla; DOCA, deoxycorticosterone acetate; ERK, extracellular-signal-regulated kinase; NE, noradrenaline; NET, NE transporter; NOS, NO synthase; nNOS, neuronal NOS; NTS, nucleus tractus solitarii; PKG, cGMP-dependent protein kinase; PVN, paraventricular nucleus; RAS, renin–angiotensin system; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rat; Stx2, Shiga toxin type 2; TH, L-tyrosine hydroxylase

Correspondence: Professor Mariela M. Gironacci (email mariela@qb.ffyb.uba.ar).

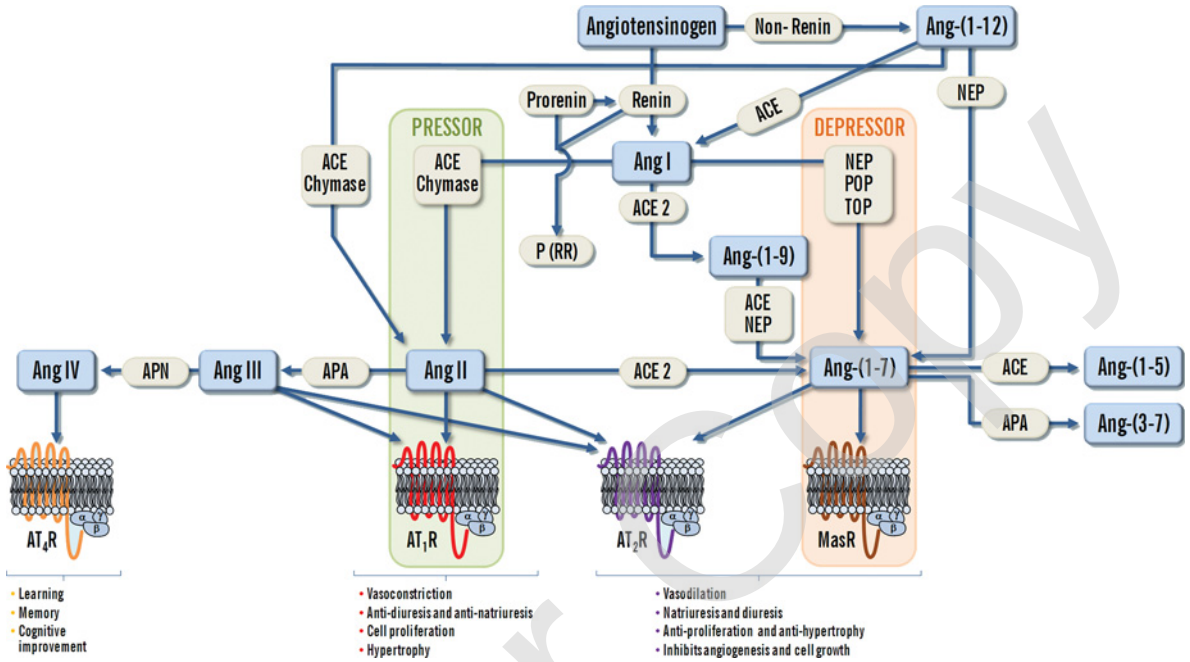

Figure 1 **Pathways involved in angiotensin peptide formation**
Ang, angiotensin; NEP, neutral endopeptidase (neprilysin); TOP, thimet oligopeptidase; POP, prolyl endopeptidase; APA, aminopeptidase A; APN, aminopeptidase N; (P) RR, prorenin receptor; AT_4R, angiotensin type 4 receptor; MasR, Mas receptor.

CENTRAL EFFECTS OF ANG-(1–7)

The first evidence *in vivo* showing that Ang-(1–7) is a biologically active neuropeptide was demonstrated by Campagnole-Santos et al. [12], who showed that unilateral injections of Ang-(1–7) into the medial NTS (nucleus tractus solitarii) or into the dorsal motor nucleus of the vagus caused depressor and bradycardic effects. Together with the findings that most neurons in the PVN (paraventricular nucleus) of anaesthetized rats are excited after micro-iontophoretic application of the heptapeptide into this area [13], that Ang-(1–7) increases neuron firing rate in the isolated canine medulla [14] and that it stimulates vasopressin secretion from rat hypothalamic brain explants [15] led to the suggestion that Ang-(1–7) acts as a central neuropeptide.

In contrast with AngII, Ang-(1–7) has been shown to improve the baroreflex control of heart rate in normotensive rats and SHRs (spontaneously hypertensive rats) [16,17] and in conscious rabbits with heart failure [18]. Ang-(1–7) enhanced the baroreflex control of heart rate by a profound effect on vagal outflow [18]. The attenuated counterbalancing effect of Ang-(1–7) on baroreflex function is lost in older rats, which may be attributable to diminished production of the peptide [19]. Supporting the role of Ang-(1–7) on baroreflex control of heart rate, Xiao et al. [20] have shown that brain-selective overexpression of ACE2 enhances baroreflex function in chronic heart failure through attenuating sympathetic outflow. This response involves Ang-(1–7) action. An ACE2 inhibitor injected into the NTS attenuated the function of the baroreflex for heart rate control in response to increases in arterial pressure, suggesting that local synthesis of Ang-(1–7) from AngII is required for normal sensitivity for the baroreflex control of heart rate [21]. Moreover, endogenous Ang-(1–7) has been shown to be involved in the improvement of baroreflex sensitivity observed in SHRs [22] and in a model of renovascular hypertensive rats [23] during central ACE inhibition, that is during AngII generation blockade, suggesting that Ang-(1–7) may contribute to the beneficial effects of ACE inhibitors widely used in the antihypertensive therapy. Recently, it has been shown that intracerebroventricular infusion of Ang-(1–7) in DOCA (deoxycorticosterone acetate)-salt hypertensive rats lowered mean arterial pressure and normalized the baroreflex control of arterial pressure and the cardiac autonomic tone [24], suggesting that increased availability of Ang-(1–7) in the brain may have an important impact on attenuating the development of DOCA-salt hypertension.

Ang-(1–7) acts at sites involved in the control of cardiovascular function, thus contributing to blood pressure regulation (Figure 2). When Ang-(1–7) was injected into the NTS [12], CVLM (caudal ventrolateral medulla) [25–27], PVN [13,28–30] or anterior hypothalamic area [31], a reduction in blood pressure occurred. In addition, microinjection of Ang-(1–7) into the PVN increases renal sympathetic nerve activity with a magnitude similar to that observed after an equimolar injection of AngII [32]. Recently, Gomez da Silva et al. [33] have shown in a rat transgenic model with low levels of brain angiotensinogen and consequently with largely reduced AngII and Ang-(1–7) levels that the sympathetic outflow mediated by PVN neurons is suppressed, corroborating the functional significance of brain angiotensin production in the central regulation of sympathetic output to the cardiovascular system.

Ang-(1–7) injected into the anterior hypothalamic area of SHRs induced a decrease in blood pressure [34]. This lowering

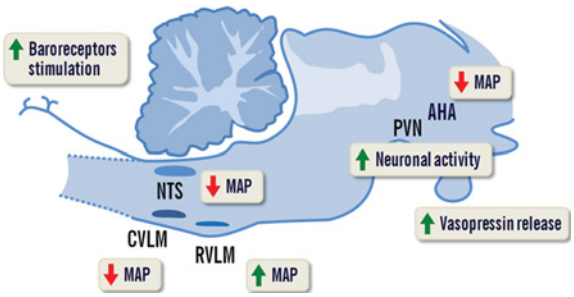

Figure 2 Effects of Ang-(1–7) on different areas of the CNS associated with blood pressure regulation

Arrows indicate an increased (↑) or reduced (↓) effect. Note: the effect on vasopressin release has been shown *in vitro*. AHA, anterior hypothalamic area; MAP, mean arterial pressure.

blood pressure effect caused by Ang-(1–7) was blunted when NO generation was inhibited [34]. In accordance, the hypotensive response of Ang-(1–7) in the CVLM was also abolished by NOS (NO synthase) inhibitors [35]. In fact, Ang-(1–7) induced an increase in NOS activity and nNOS (neuronal NOS) expression in hypothalami from SHRs [34]. Yang et al. [36] have shown that stimulation of differentiated catecholaminergic neurons with Ang-(1–7) caused an increase in NO generation through nNOS activation, which in turn activates neuronal potassium currents. Reinforcing the stimulatory action of Ang-(1–7) on NO generation, Feng et al. [37] have shown that central ACE2 overexpression reverses neurogenic hypertension, partially by preventing the decrease in both spontaneous baroreflex sensitivity and parasympathetic tone. Ang-(1–7) plays a pivotal role in this reversal, promoting NOS activation and leading to enhanced NO release in the CNS (central nervous system) [37].

In contrast, an increase in blood pressure was observed when the peptide was injected into the rat RVLM (rostral ventrolateral medulla) [26,27,38,39]. The pressor effect caused by Ang-(1–7) in the RVLM was greater in SHRs than in Wistar–Kyoto rats [39]. The RVLM plays a pivotal role in regulating sympathetic vasomotor activity. Ang-(1–7), when injected into the RVLM, is as effective as AngII in enhancing cardiac sympathetic afferent reflex, which results in an increase in renal sympathetic nerve activity and mean blood pressure [38]. In contrast with the rat, Ang-(1–7) does not seem to have a biologically significant action in the ventrolateral medulla of the rabbit [40]. Microinjections of Ang-(1–7) into the RVLM and CVLM of rabbits evoked dose-dependent increases and decreases respectively in arterial pressure and renal sympathetic nerve activity, but in comparison with AngII much higher doses (approximately 50-fold higher) were required to produce cardiovascular response of a similar magnitude [40].

In contrast with the effect on blood pressure regulation reported for Ang-(1–7) centrally (see above), Jiang et al. [41] have shown that Ang-(1–7) administration into the right lateral cerebral ventricle over 4 weeks did not modify blood pressure, but remarkably inhibited RAS activity by reducing cerebral AngII and AT_1 (angiotensin type 1) receptor levels, diminishing oxidative stress levels and attenuating neuronal apoptosis in the brains of SHRs.

Ang-(1–7) not only contributes to central blood pressure regulation by itself, but also by blockade of the AngII pressor effect. Intrahypothalamic injections of Ang-(1–7) blocked the pressor response elicited by AngII in SHRs [42] and in sinoaortic denervated rats [31]. In contrast, ACE2 overexpression in the subfornical organ impairs AngII-mediated pressor response, but this reduction in the AngII pressor response was independent of Ang-(1–7) [43].

Importantly, Ang-(1–7) has been shown to be cerebroprotective during ischaemic stroke. Intracerebroventricular infusion of Ang-(1–7) significantly attenuated the cerebral infarct size and neurological deficits elicited by ET (endothelin)-1-induced middle cerebral artery occlusion, a model of cerebral ischaemia [44]. This protective action of Ang-(1–7) includes blunting of iNOS (inducible NOS) expression [44].

Ang-(1–7) not only acts as a cerebroprotective agent during ischaemia, but also prevents neurological damage induced by Stx2 (Shiga toxin type 2). Shiga toxin from enterohemorrhagic *Escherichia coli* is the main cause of haemorrhagic colitis, which may result in haemolytic–uraemic syndrome [45]. Intracerebroventricular administration of Stx2 induces apoptosis in neurons, glial ultrastructural alterations and demyelinated fibres in the rat corpus striatum [46]. In accordance, injections of Stx2 into the rat anterior hypothalamic area induced neuronal alterations and demyelinated fibres. As shown in Figure 3(A), hypothalamic neurons from the vehicle-treated group showed pale nuclei and well-dispersed chromatin, intact cytoplasmic and nuclear membranes, and intact dispersed mitochondria in the cytoplasm. In contrast, neurons from the Stx2-treated group showed a degenerative condition, with vacuolated cytoplasm and indentations in the nuclear membrane, disorganized axons, and a lower amount of axons with reduced myelin thickness (Figures 3B and 3E), and this effect was prevented when Stx2 was co-administered with Ang-(1–7) (Figures 3C and F), reinforcing the cerebroprotective role of Ang-(1–7) (M.M. Gironacci and J. Goldstein, unpublished work).

Besides its effects on cardiovascular function, Ang-(1–7) has been shown to enhance long-term potentiation in the CA1 region of the hippocampus [47] and in the lateral amygdala of rats [48]. Long-term potentiation and long-term depression are two forms of activity-dependent synaptic plasticity, which are considered to be involved in learning and memory. In addition, Ang-(1–7) has been shown to be involved in object recognition memory function [49].

RECEPTORS MEDIATING ANG-(1–7) EFFECTS

The Mas proto-oncogene, originally considered to be an 'orphan' G-protein-coupled receptor associated with phospholipase C activation [50], has been shown to specifically bind Ang-(1–7) and is involved in many of its biological actions [8,51]. At the central level, a strong staining for the Mas receptor was found in the NTS, CVLM and RVLM, inferior olive, parvocellular and magnocellular portions of the paraventricular hypothalamic nucleus, supraoptic nucleus and lateral pre-optic area [52], areas where centrally

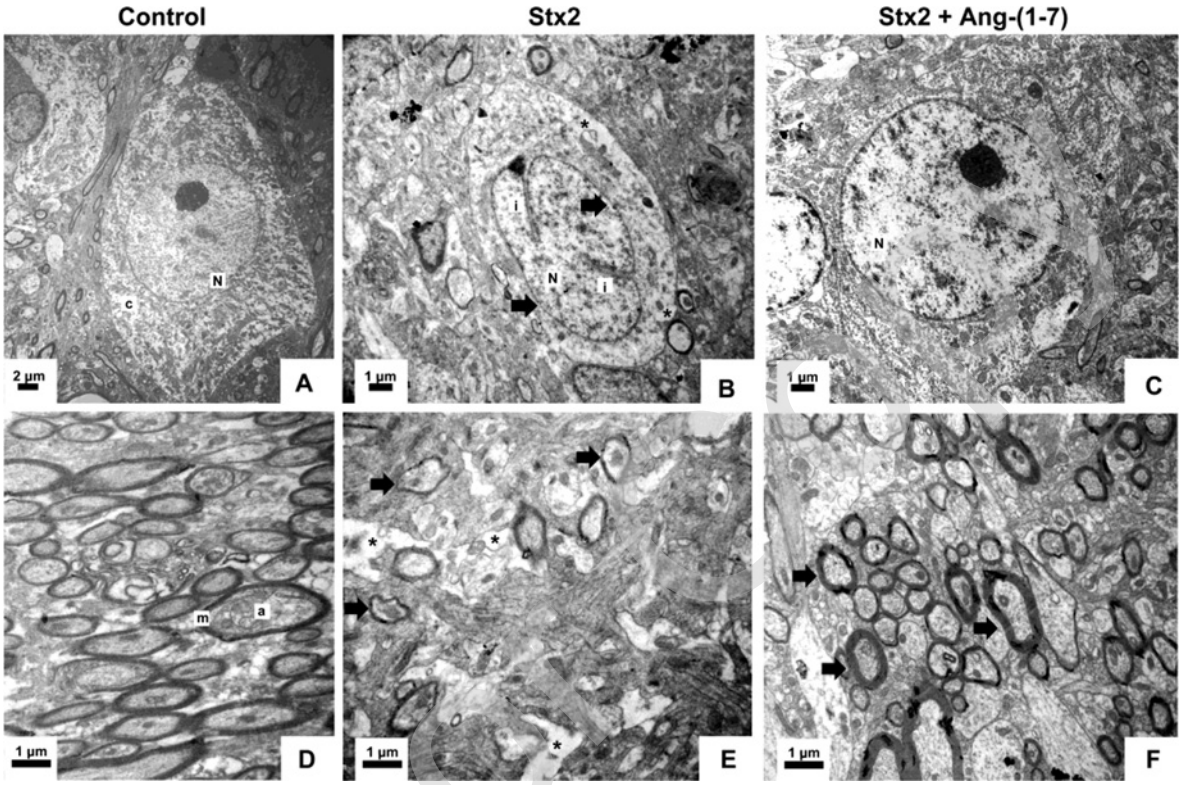

Figure 3 **Ang-(1–7) prevents neuronal and axonal damage induced by Stx2**
Rats received intrahypothalamic injections of saline (Control), Stx2 (10 ng) or Stx2 + Ang-(1–7) (50 ng) over 8 days. Structural changes were analysed by transmission electron microscopy. Hypothalamic neurons and axons are shown in (**A**–**C**) and (**D**–**F**) respectively. A regular pale nucleus (N) and intact cytoplasm (∗) were observed under control conditions (**A**). After Stx2 treatment, the cytoplasm becomes vacuolated (c) and the nucleus (N) shows prominent nuclear indentation (i) with partial loss of membranes (arrows) (**B**). Administration of Ang-(1–7) prevents Stx2-induced neuronal damage (**C**). Axons were well surrounded by myelin sheaths (m) under control conditions (**D**). After Stx2 treatment, axonal degeneration was observed. Oedematous parenchyma was accompanied by disorganized axons and a lesser amount of axons with reduced myelin thickness (**E**). Ang-(1–7) prevented the axonal degeneration induced by Stx2 (**F**). No oedema was observed in the parenchyma, and healthy axons display conserved myelin (m) after Ang-(1–7) treatment (**F**). This Figure is from unpublished work by M.M. Gironacci and J. Goldstein.

Ang-(1–7) effects have been shown. Furthermore, Mas receptor staining was predominantly present in neurons [52]. Mas receptors are present in neurons from hypothalamus and brainstem of Wistar–Kyoto rats and SHRs, its being expression higher in SHRs [53]. At the medullary sites, in central areas related to blood pressure regulation, a specific and high-affinity binding to Ang-(1–7) has been shown [52]. This binding was completely displaced by an anti-Mas antibody or by [D-Ala^7]Ang-(1–7), the specific Ang-(1–7) antagonist, demonstrating that Ang-(1–7) binds to central Mas receptors [52].

Prolonged stimulation of the Mas receptor with Ang-(1–7) or with high concentrations of the ligand caused an attenuation of receptor responsiveness [54,55], suggesting receptor desensitization. Receptor desensitization represents an important physiological 'feedback' mechanism that protects against both acute and chronic receptor overstimulation and is the consequence of receptor internalization. The spatial and temporal control of receptors determines the specificity of receptor-mediated signal transduction among the distinct downstream effectors and the ultimate cellular response. We have shown that Mas receptors are internalized upon Ang-(1–7) stimulation into early endosomes via a clathrin-dependent pathway; however, at some point in the receptor trafficking Mas receptors traverse caveolin-1-positive compartments [56], suggesting that Mas receptors are also internalized through caveolae. After being internalized, Mas receptors are recycled back to plasma membrane through slow recycled vesicles to be active again (M.M. Gironacci and J. Goldstein, unpublished work).

Despite the fact that many central biological actions of Ang-(1–7) are mediated by Mas receptors, Ang-(1–7) at micromolar concentrations acts as a weak agonist at the AT_1 receptor in vascular smooth muscle cells and down-regulates AT_1 receptors by reducing the total number of binding sites for AngII [57]. Diz and Ferrario [58] have demonstrated that Ang-(1–7) in the CNS is able to compete for almost all the specific AngII-binding sites in the rostral, but not in the caudal, region of the dorsal medulla nuclei, with a 30–40-fold less potency than AngII. A great percentage of the AngII receptors in the rostral part of the dorsomedial medulla are AT_2 (angiotensin type 2) subtype, which suggests that Ang-(1–7) binds to AT_2 receptors [59]. In fact, several reports indicate that diverse central effects of Ang-(1–7) are impaired by AT_2 receptor antagonists, i.e. the Ang-(1–7)-induced neuronal

excitation in the PVN [13], the prostaglandin release in astrocytes [60], the phosphoinositide turnover enhancement in neonatal rat brain [61], NE [noradrenaline (norepinephrine)] release from the hypothalamus of normotensive rats and SHRs [62,63] or TH (L-tyrosine hydroxylase) expression, the rate-limiting enzyme in NE biosynthesis, in neuronal cultures from the hypothalamus of SHRs [64]. These findings suggest an interaction of Ang-(1–7) with AT_2 receptors.

ANG-(1–7) AND NE NEUROMODULATION

A large number of neurotransmitters contribute to regulating sympathetic outflow. The modulatory effects of AngII or Ang-(1–7) in the CNS are, to some extent, a result of actions on synaptic transmission. NE release from the hypothalamic nuclei contributes to blood pressure regulation by altering sympathetic nervous system activity [65,66]. A central disturbance in NE release by the hypothalamus is related to increased sympathetic nervous system activity, which contributes to high blood pressure in hypertension [66–68].

One of the physiological mechanism by which AngII exerts its pressor effect involves the modulation of sympathetic activity and the regulation of catecholamine metabolism in the brain nuclei typically associated with the control of blood pressure [66,69]. Central AngII administration enhances NE release in the PVN in parallel with the pressor response, and increases TH mRNA and activity in the hypothalamus and brainstem [69]. In addition, central catecholaminergic depletion or noradrenergic antagonist administration prevents the pressor response to central AngII, indicating that the AngII-induced pressor response is mediated by NE [69].

In contrast, we have shown previously that Ang-(1–7) attenuates the potassium-evoked neuronal NE release in hypothalami from normotensive rats and SHRs, this effect being greater in SHRs [62,63]. The inhibitory effect induced in rat hypothalamus differs from that produced in the peripheral nervous system, in which the heptapeptide acts presynaptically, increasing NE released by nerve stimulation [70,71]. Thus Ang-(1–7) has a tissue-specific neuromodulatory effect on noradrenergic neurotransmission, being inhibitory at the central level. In accordance, Xiao et al. [20] have shown that brain-selective overexpression of ACE2 decreases NE urinary excretion and renal sympathetic nerve activity through Ang-(1–7) and Mas receptor signalling, reinforcing the neuroinhibitory role of Ang-(1–7) on sympathetic neurotransmission.

Centrally, Ang-(1–7) decreases NE release through a NO-related mechanism [62,63]. Thus Ang-(1–7) acting through its receptors Mas or AT_2 receptors induces intracellular generation of bradykinin, which binds to B_2 receptors with the consequent activation of NOS, increasing in this way NO production [63]. NO stimulates soluble guanylate cyclase with the consequent increase in cytosolic cGMP concentration, which leads to activation of PKG (cGMP-dependent protein kinase) [63] (Figure 4). PKG, probably by phosphorylation of either voltage-dependent calcium channels, resulting in their inhibition, or of synaptic vesicle proteins associated with neurotransmitter release, induces a low NE outflow. Thus a bradykinin/NO mechanism plays a critical role in the inhibition of NE release caused by Ang-(1–7) [63]. Supporting the role of NO in mediating the Ang-(1–7) effect, it has been shown that Ang-(1–7) induces NO generation in differentiated catecholaminergic neurons [36].

The neuromodulatory inhibitory action of Ang-(1–7) on NE release may also result from a blocking action on the increased NE release elicited by AngII. In accordance with this possibility, we have shown previously that Ang-(1–7) not only diminished NE release, but also blocked AngII-enhanced NE outflow from hypothalami isolated from hypertensive rats with aortic coarctation through AT_2 receptors [72]. Receptor activation by Ang-(1–7) results in a bradykinin-dependent stimulation of NO release, which in turn inhibits the enhanced NE release caused by AngII [72]. Recently, Feng et al. [73] have shown that ACE2 overexpression in brain decreases urine NE levels in response to AngII.

NE is synthesized from tyrosine as a precursor and packed into synaptic vesicles. It performs its action by being released into the synaptic cleft, where it acts on adrenergic receptors, followed by the signal termination, either by degradation of NE or by uptake by surrounding cells (Figure 4). Noradrenergic neurotransmission is a complex mechanism that includes a signal reception followed by its interpretation and processing to produce a response. The neurotransmitter is key in this mechanism, so its synthesis, storage, release, interaction with specific receptors, removal from the synaptic cleft and catabolism are highly regulated. Ang-(1–7) not only acts as a negative modulator of central NE by reducing its release, but also by modulating neurotransmitter synthesis. Ang-(1–7) decreases the activity and expression of TH, the enzyme that catalyses the first and rate-limiting step in catecholamine biosynthesis, in hypothalami and neuronal cultures from the hypothalamic-brainstem of normotensive rats and SHRs [64]. Ang-(1–7) diminishes TH enzymatic activity by reducing its phosphorylation [64]. TH activity can be regulated by protein phosphorylation at serine residues by a variety of protein kinases [74]. Phosphorylation of TH at Ser^{19} has no effect on TH activity, but phosphorylation at Ser^{19} alters the conformation of TH to allow increased accessibility of Ser^{40} to kinases, leading to an increase in the enzyme activity [74]. Ang-(1–7) decreases TH phosphorylation at Ser^{19} and Ser^{40} in hypothalamic-brainstem neuronal cultures from normotensive Wistar–Kyoto rats and SHRs [64].

Ang-(1–7) also down-regulates TH by increasing its degradation, and this effect is coupled to AT_2 receptor stimulation [64]. The ubiquitin–proteasome system is a multicatalytic protease complex found in almost all living cells and is responsible for selective protein degradation in the cytoplasm [75,76]. Degradation of a protein via this system involves covalent conjugation of ubiquitin to the target protein, followed by its recognition by the 26S proteasome and degradation of the polyubiquitinated protein into small peptides [75,76]. Ang-(1–7)-induced down-regulation of TH expression in neuronal cultures from SHRs is mediated by ubiquitin–proteasome pathway stimulation (Figure 4). Thus Ang-(1–7) stimulates both ubiquitin conjugation to TH and its subsequent proteasome recognition, which in turn increases TH degradation, leading to a decreased cellular content of TH [64],

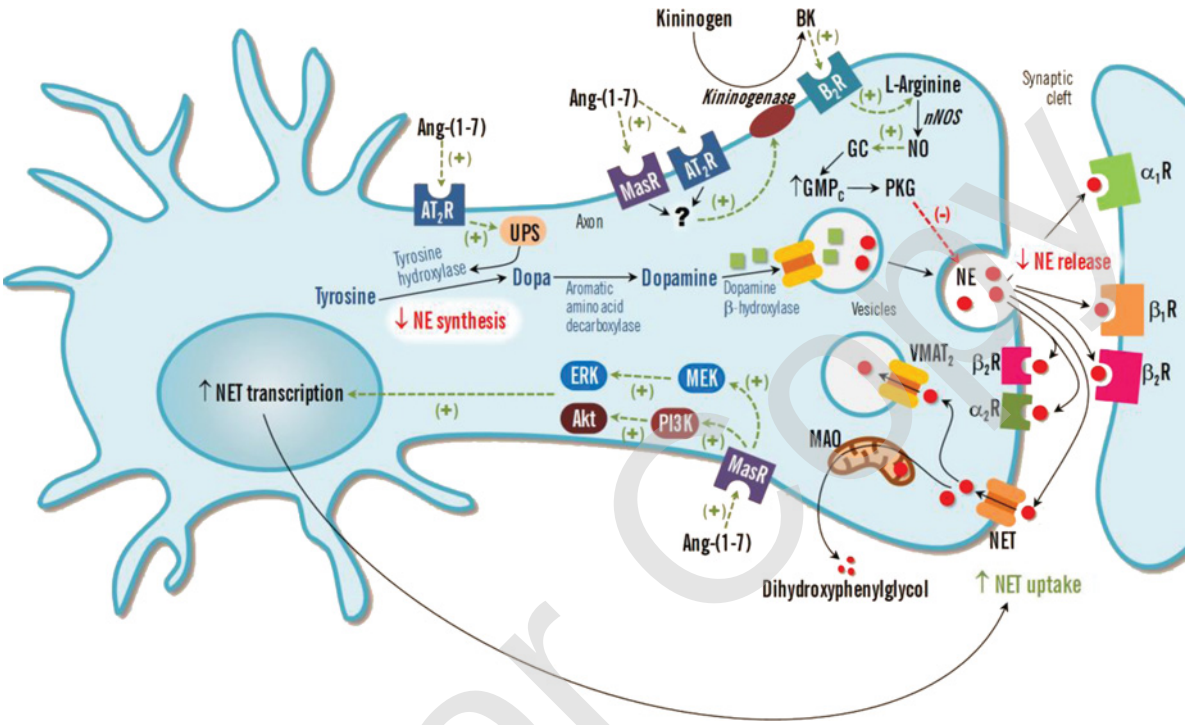

Figure 4 **Schematic representation of a sympathetic neuron and the targets of Ang-(1–7) action, which results in changes in NE levels in the synaptic cleft**

α_1R etc., α_1 receptor etc.; MasR, Mas receptor; B_2R, bradykinin B_2 receptor; BK, bradykinin; UPS, ubiquitin–proteasome system; GC, guanylate cyclase; PI3K, phosphoinositide 3 kinase.

demonstrating a novel mechanism of action for Ang-(1–7) to down-regulate a protein involved in the development of hypertension.

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 [77]. NET (NE transporter) regulates noradrenergic signalling in the CNS and peripheral nervous systems by mediating the clearance of NE fine-tuning neurotransmitter levels at the synaptic cleft. Altered pattern or a disturbance in the pre- and post-synaptic regulatory mechanisms could lead to abnormal neurotransmission and hence abnormal behaviour, brain disorders or hypertension [77,78]. An increased rate of sympathetic nerve firing and reduced neuronal NE reuptake both contribute to sympathetic activation in hypertension [79]. Ang-(1–7) does not evoke an acute but a long-term effect on NE neuronal uptake in hypothalami from SHRs by increasing NET expression in hypothalamic and brainstem neurons [53]. The Ang-(1–7)-stimulated NET expression in hypothalamic and brainstem neurons from SHRs is coupled with Mas receptor activation acting through both MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase) kinase] 1/2–ERK1/2 and PI3K (phosphoinositide 3-kinase)–Akt-dependent pathways [53] (Figure 4). When endogenous Ang-(1–7) was blocked with a specific antibody against Ang-(1–7), a decrease in NE neuronal uptake, as well as in NET expression, was observed [53], reinforcing the stimulatory role of Ang-(1–7) on NE uptake. Taken together, these results confirm the long-term sympatoinhibitory effect of Ang-(1–7) via regulation of NET expression. In this way, Ang-(1–7) regulates a presynaptic mechanism in maintaining appropriate synaptic NE levels during hypertensive conditions.

The fact that Ang-(1–7) inhibits NE synthesis and release and increases NET expression, as well as NE uptake in neurons, supports a negative neuromodulatory role for Ang-(1–7) on central sympathetic nervous system activity, thus contributing to the modulation of NE homoeostasis. In fact, it has been shown that central ACE2 overexpression exerts a potential protective effect on cardiac heart failure through attenuating sympathetic outflow, implicating Ang-(1–7) and Mas receptors in this action [20], and reinforcing the role of Ang-(1–7) as an inhibitory NE neuromodulator.

ANG-(1–7) AND OTHER NEUROTRANSMITTERS

Substance P is a small excitatory peptidic neurotransmitter that, when introduced into lateral ventricles and some hypothalamic nuclei including the PVN, increases blood pressure and activates the sympathetic nervous system [66]. Substance P is present in vagal sensory and motor nerves and is a potential

transmitter/facilitatory modulator of the baroreflex [80]. Low doses of AngII injected into the NTS or dorsal motor nucleus of the vagus reduces blood pressure and heart rate, which mimics baroreflex activation. This effect is mediated by substance P release, demonstrating that alterations in substance P pathways at the hypothalamus may contribute to the actions of AngII on reflex function [81]. In contrast, Ang-(1–7) fails to modify substance P release in perfused rat medulla slices, but facilitates a potassium-evoked release of substance P in isolated rat hypothalamus [82].

Glutamate, an excitatory neurotransmitter amino acid, and taurine, an inhibitory amino acid, play important roles in regulating both tonic and reflex cardiovascular activity at the CVLM [83]. Wang et al. [84] have shown that the depressor response caused by Ang-(1–7) after injection into the CVLM is accompanied by an increased release of glutamate and a decrease of taurine. Conversely, [D-Ala^7]Ang-(1–7), the specific Ang-(1–7) antagonist, has opposing activity on transmitter release [84]. These data suggest that Ang-(1–7) may modulate the release of glutamate and taurine at the CVLM, which in turn contribute at least in part to the hypotensive effect of Ang-(1–7).

CONCLUSIONS

Fluctuations in neurotransmitter levels in brain areas involved in cardiovascular regulation lead to changes in blood pressure and/or isovolaemia. Blood pressure homoeostasis is maintained with the participation of several brain regions and neurotransmitters which possess similar or opposing functions when released from CNS neurons. Thus, by selective neurotransmitter synaptic level modulation, Ang-(1–7) may contribute to the overall central cardiovascular effects.

The actual view of the RAS is that composed of two opposing arms: (i) the pressor arm constituted by the enzyme ACE, AngII as the product, and the AT_1 receptor as the main protein mediating the biological actions of AngII; and (ii) the depressor arm composed of ACE2, Ang-(1–7) produced through hydrolysis of AngII, and the Mas receptor as the protein mediating the vasodilator, depressor, natriuretic, cardioprotective, antiproliferative, antifibrotic and antithrombotic effects of Ang-(1–7) [7,8] (Figure 1). Taking into account the protective role of Ang-(1–7) at the central level and its balancing effect on AngII actions, one should consider the depressor arm of the RAS as a potential therapy. That is, we should not only consider blocking the pressor arm, but also potentiating the activity of the depressor arm of the RAS. Further exploration on the molecular basis of Ang-(1–7) actions and how Mas receptors are regulated would open new therapeutical possibilities.

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