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Review Article

Protective axis of the renin–angiotensin system in the brain

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Abstract

The RAS (renin–angiotensin system) is composed of two arms: the pressor arm containing AngII (angiotensin II)/ACE (angiotensin-converting enzyme)/AT_1Rs (AngII type 1 receptors), and the depressor arm represented by Ang-(1–7) [angiotensin-(1–7)]/ACE2/Mas receptors. All of the components of the RAS are present in the brain. Within the brain, Ang-(1–7) contributes to the regulation of BP (blood pressure) by acting at regions that control cardiovascular function such that, when Ang-(1–7) is injected into the nucleus of the solitary tract, caudal ventrolateral medulla, paraventricular nucleus or anterior hypothalamic area, a reduction in BP occurs; however, when injected into the rostral ventrolateral medulla, Ang-(1–7) stimulates an increase in BP. In contrast with AngII, Ang-(1–7) improves baroreflex sensitivity and has an inhibitory neuromodulatory role in hypothalamic noradrenergic neurotransmission. Ang-(1–7) not only exerts effects related to BP regulation, but also acts as a cerebroprotective component of the RAS by reducing cerebral infarct size and neuronal apoptosis. In the present review, we provide an overview of effects elicited by Ang-(1–7) in the brain, which suggest a potential role for Ang-(1–7) in controlling the central development of hypertension.

Key words: angiotensin-(1–7), angiotensin-converting enzyme 2 (ACE2), baroreflex, hypertension, Mas receptor, noradrenergic neurotransmission, renin–angiotensin system (RAS)

ANGIOTENSIN-(1–7) GENERATION AND DISTRIBUTION IN THE BRAIN

The RAS (renin–angiotensin system) is a hormonal cascade involved in arterial pressure and fluid homoeostasis, and cardiovascular function regulation. Deregulation of the RAS plays an important role in the pathogenesis of cardiovascular diseases [1,2]. It is well known that not only a systemic RAS, but also a tissue and even an intracellular RAS exist [3,4].

Ang-(1–7) [angiotensin-(1–7)] is generated from AngI (angiotensin I) through an ACE (angiotensin-converting enzyme)-independent pathway. Neutral endopeptidase (EC 3.4.24.11; EP 24.11, neprilysin), thimet oligopeptidase (EC 3.4.24.15) and prolyl oligopeptidase (EC 3.4.21.26) have been reported to be involved in the central generation of Ang-(1–7) from AngI [5–7] (Figure 1). In 2000, a new enzyme was described, ACE2, and this was shown to be involved in Ang-(1–7) generation. ACE2 is a carboxypeptidase that converts AngI into Ang-(1–9) [angiotensin-(1–9)], which is subsequently cleaved to Ang-(1–7) by ACE or neutral endopeptidase enzymatic activities [8,9] (Figure 1). Later, it was shown that ACE2 displays more affinity for AngII, yielding Ang-(1–7) with a catalytic efficiency 400-fold greater for AngII than for AngI [10] (for a detailed enzymatic pathways for the brain RAS, see [7]).

In the mouse brain, ACE2 is widespread in areas both involved in the regulation of cardiovascular function and also those that are not [11]. The OVLT (organum vasculosum of the lamina terminalis), an area involved in thirst and salt appetite, or brain nuclei involved in the regulation of cardiovascular function, such as the SFO (subfornical organ), the magnocellular neurons of the PVN (paraventricular nucleus), the area postrema, the dorsal motor nucleus of the vagus, the NTS (nucleus of tractus solitarii), the

Abbreviations: ACE, angiotensin-converting enzyme; ADAM, a disintegrin and metalloproteinase; Ang-(1–7), angiotensin-(1–7); AngI etc., angiotensin I etc.; AT_1R etc., AngII type 1 receptor etc.; Beta, betamethasone; BP, blood pressure; CHF, chronic heart failure; COX2, cyclo-oxygenase 2; CVLM, caudal ventrolateral medulla; DOCA, deoxycorticosterone acetate; ERK, extracellular-signal-regulated kinase; GABA, γ-aminobutyric acid; IL, interleukin; MAP, mean arterial pressure; NOS, NO synthase; NTS, nucleus of tractus solitarii; OVLT, organum vasculosum of the lamina terminalis; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PVN, paraventricular nucleus; RAS, renin–angiotensin system; RVLM, rostraventrolateral medulla; sACE2, soluble ACE2; SFO, subfornical organ; SHR, spontaneously hypertensive rat; TNF, tumour necrosis factor.

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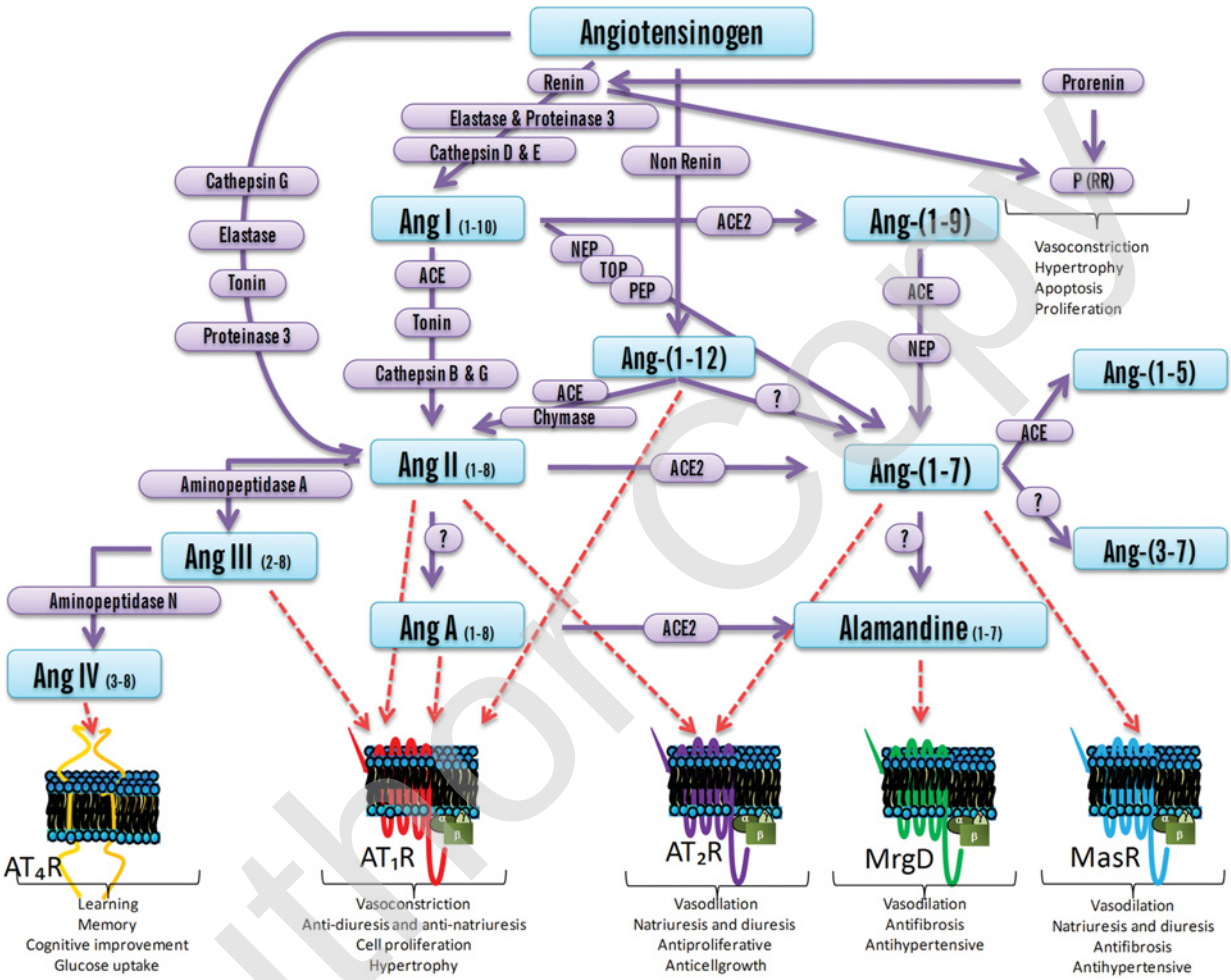

Figure 1 **Brain RAS**
Abbreviations: Mas R, Mas receptor; MrgD, Mas-related G-protein-coupled receptors; NEP, neutral endopeptidase (neprilysin); PEP, prolyl endopeptidase; (P)RR, prorenin receptor; TOP, thimet oligopeptidase. This Figure was adapted and modified from [29] with permission. © 2013 Biochemical Society.

RVLM (rostroventrolateral medulla) and the nucleus ambiguus all show positive staining for ACE2 (for a detailed localization of ACE2 in the brain, see [11]). Using cell-type specific antibodies, it was shown that ACE2 is present in the cytoplasm of neurons, but not in glial cells, of the mouse brain [11]. Conversely, ACE2 gene expression in cultured astrocytes isolated from neonatal rat cerebellum or medulla oblongata has been reported [12].

Immunostaining for Ang-(1–7) has been observed in areas of the brain related to hydroelectrolytic balance, including the supra-optic and PVN of the hypothalamus [13]. Consistent with this observation, Ang-(1–7) immunoreactivity was reported in neurons from the hypothalamus and brainstem [14] and in the PVN [15] of rats. In extracts from the rat hypothalamus, approximately equimolar amounts of Ang-(1–7), AngII and AngI were detected [16]. A similar profile was observed in the medulla oblongata and amygdala, although the content of these three peptides was 40–70% lower than that determined in the hypothalamus [16].

Recently, it has been shown that Ang-(1–7) is generated in the rat hippocampus, with thimet oligopeptidase being the main enzyme responsible in its generation [17]. Interestingly, Ang-(1–7) was the preferred peptide generated from AngI metabolism in hippocampal extracts from rats [17]. Furthermore, elevations in the levels of thimet oligopeptidase and Ang-(1–7) were observed in the hippocampus of epileptic rats [17]. Consistent with these findings, increased Ang-(1–7) levels in the hippocampus of rats during the acute and silent phases of pilocarpine-induced epilepsy have been reported [18].

Ang-(1–7) induces its effects mainly through Mas receptor activation, although it can also act through AT_2Rs (AngII type 2 receptors). Mas receptors were first described to be specific for Ang-(1–7) by Santos et al. [19] in 2003. With regards to localization, Mas receptors have been shown to be present in the hippocampus, amygdala, cortex and hypoglossal nucleus, as well as in the cardiovascular-related areas of the medulla and forebrain [20]. A strong immunostaining was observed in the NTS, CVLM (caudal ventrolateral medulla) and RVLM, inferior olive, PVN and in the supra-optic nucleus [20]. Mas receptor staining was predominantly present in neurons [20]. Mecca et al. [21]

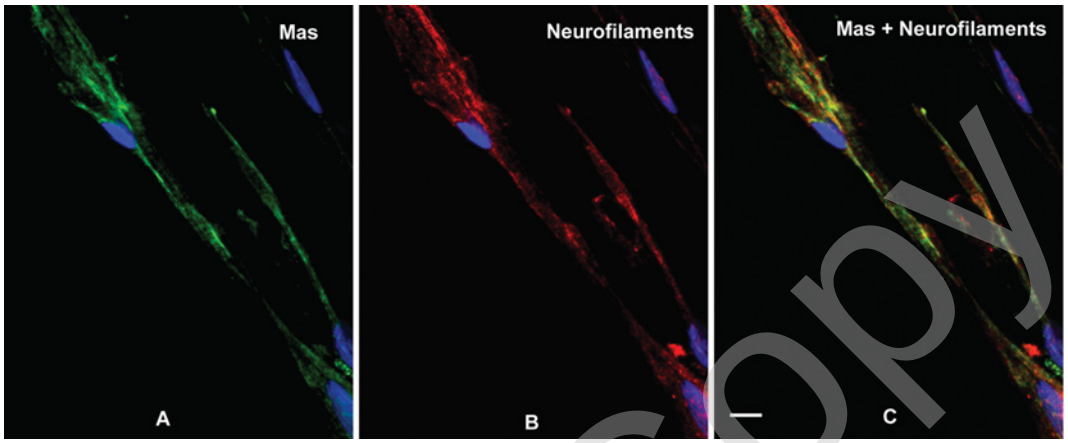

Figure 2 **Mas receptor in neurons**

(A) Mas receptor immunoreactivity (green), (**B**) neurofilament immunoreactivity (red) and (**C**) Mas receptor plus neurofilament co-localization in neuronal cultures from the hypothalamus and brainstem of newborn rats. After fixation, cells were incubated overnight with antibodies against Mas receptor (1:200 dilution) and neurofilaments (1:300 dilution), a neuronal marker, followed by the secondary antibodies Alexa Fluor® 510 and Alexa Fluor® 594 (1:400 dilution). Non-specific staining was determined in the absence of the primary antibodies. Images were taken using an Olympus Fluoview FV1000 spectral laser scanning confocal microscope with a ×60 oil-immersion lens. Scale bar, 5 μm. F.M. Cerniello and M.M. Gironacci, unpublished work.

have shown Mas immunostaining mainly in the soma of cerebral cortex neurons in rats, but not in astroglia, and to exist in both non-nuclear and nuclear compartments [21]. In primary neuronal cultures from the hypothalamus and brainstem of newborn rats, we have observed Mas receptor co-localization with neurofilaments, demonstrating the presence of Mas receptors in neurons (F.M. Cerniello and M.M. Gironacci, unpublished work) (Figure 2). In addition, Mas receptor protein expression was greater in neurons from the hypothalami of SHRs (spontaneously hypertensive rats) than from normotensive Wistar–Kyoto rats [22]. Further immunostaining experiments have revealed the presence of Mas receptors on macrophages/microglia in the rat cerebral cortex, as shown by co-localization with a specific marker of these cells [21].

Within the mouse brain, the strongest Mas receptor protein expression was detected in the dentate gyrus of the hippocampus and within the piriform cortex [23]. However, Mas receptor protein expression is not restricted to these areas, as Mas-receptor-immunopositive neurons were also observed in different parts of the cortex, hippocampus, amygdala, basal ganglia, thalamus and hypothalamus [23].

Taken together, these findings provide an anatomical basis for the physiological role of Ang-(1–7) at the central level within the brain.

Ang-(1–7) AND BLOOD PRESSURE REGULATION

Studies investigating the biological role of Ang-(1–7) in the brain began about 25 years ago. In 1998, Campagnole-Santos et al. [24] reported that Ang-(1–7) had depressor and bradycardic effects *in vivo* when injected into the NTS or the dorsal motor nucleus of the vagus, thus showing that this peptide was biologically active within the brain. The NTS is the main termination site of primary afferent fibres arising from many cardiovascular receptors. It receives inputs from nuclei at all levels of the brain and innervates medullary, as well as supramedullary, centres. Several of these interconnected centres within the central nervous system contribute to cardiovascular homeostasis by adjusting BP (blood pressure) [25,26]. However, in transgenic rats with a severe deficit of brain angiotensinogen production, the depressor and bradycardic response caused by Ang-(1–7) was attenuated, whereas that caused by AngII was unaltered, suggesting that a decrease in brain RAS activity may lead to a differential alteration in the responsiveness of angiotensin receptors [27].

Actions on arterial baroreceptors are the main mechanism for the short-term (seconds to minutes) control of MAP (mean arterial pressure) by sending afferent inputs to a medullary circuit that controls the sympathetic drive to the heart and peripheral vasculature [28]. In opposition to AngII, Ang-(1–7) has been shown to facilitate the baroreflex control of heart rate (for a review, see [29]). Accordingly, central infusion of an Ang-(1–7) antagonist blunted the baroreflex sensitivity in normotensive rats, but not in SHRs, whereas central infusion of an AT_1R (AngII type 1 receptor) antagonist facilitated the sensitivity of the baroreceptor control of heart rate in both strains [30]. These results suggest that central endogenous AngII and Ang-(1–7) differentially modulate the baroreflex through distinct receptors. Imbalances in angiotensin peptide formation and/or action may be responsible for the depressed baroreceptor reflex sensitivity in SHRs [30]. In older transgenic rats with low glial angiotensinogen, Mas receptor blockade in the NTS impaired baroreflex sensitivity, whereas AT_1R blockade induced no change, suggesting that glial angiotensinogen is the main source of AngII required for the attenuation of baroreflex sensitivity, whereas endogenous Ang-(1–7) from non-glial sources enhances baroreflex sensitivity [31].

These findings suggest a novel mechanism for the preservation of baroreflex sensitivity during aging [31].

In hypertensive (mRen2)27 transgenic rats, a model of chronically overactive brain RAS with impaired baroreflex function [32], Ang-(1–7) treatment significantly improved the vagal components of baroreflex function and heart rate variability at a dose that did not significantly lower MAP [33]. Replacement of Ang-(1–7) through gene transfer of a fusion protein that forms Ang-(1–7) in the brain of (mRen2)27 rats reverses, in part, the hypertension and baroreflex impairment observed in this model, and this is consistent with a functional deficit of Ang-(1–7) in this hypertensive strain [34]. Isa et al. [35] have shown that the improvement in baroreflex sensitivity in these transgenic rats caused by the administration of an ACE inhibitor, but not an AT_1R antagonist, into the NTS was blocked by a Mas receptor antagonist, reinforcing the role of Ang-(1–7) and the Mas receptor in baroreflex sensitivity. In agreement, Mas-receptor-knockout mice have altered cardiovascular reflex responses [36]. The lack of the Mas receptor induced an important imbalance in the neural control of BP, altering not only the baroreflex, but also the chemoreflex and Bezold–Jarisch reflex [36].

Recently, it has been shown that an imbalance in the AngII/Ang-(1–7) ratio may be responsible for the impairment in baroreflex sensitivity and heart rate variability in a sheep model of fetal programming resulting from exposure at day 80 of gestation to Beta (betamethasone) [37,38]. Beta is administered to accelerate lung development and improve survival of premature infants, but may be associated with hypertension later in life. In the Beta-exposed animals, peripheral AT_1R blockade lowered MAP and improved baroreflex sensitivity and heart rate variability, whereas Mas receptor blockade induced opposite effects: reduced baroreflex sensitivity and increased MAP. The authors concluded that Beta exposure impairs baroreflex sensitivity and heart rate variability at a time point preceding the elevation in MAP via mechanisms involving an imbalance in the AngII/Ang-(1–7) ratio, which is consistent with a progressive loss of Ang-(1–7) function [37]. In fact, fetal Beta exposure attenuates Ang-(1–7)/Mas receptor expression in the dorsal medulla of adult sheep [38].

The RVLM plays a crucial role in the tonic and phasic regulation of BP. It exerts a widespread control over the sympathetic outflow to effectors affecting cardiovascular function [25,26]. The RVLM is a major tonic pressor region which innervates directly the sympathetic pre-ganglionic neurons located in the intermediolateral cell column of the spinal cord [25,26]. The CVLM receives direct baroreceptor input from the NTS and exerts a modulatory action on RVLM neurons via a short inhibitory pathway, thus having a sympato-inhibitory action [25,26]. It has been shown that Ang-(1–7) is as effective as AngII on BP regulation when injected into the RVLM or CVLM (see [29]). Despite both peptides eliciting similar responses with respect to BP when injected into the RVLM or CVLM, differential actions on the baroreflex control of heart rate have been reported [39]. Micro-injections of AngII and Ang-(1–7) into the RVLM did not affect the baroreflex control of heart rate, whereas micro-injections of angiotensin peptides into the CVLM induced differential changes in the bradycardic or tachycardic component of the baroreflex. Although Ang-(1–7) attenuated the baroreflex bradycardia and facilitated the baroreflex tachycardia, AngII produced opposite effects. These results suggest that AngII and Ang-(1–7) produce a differential modulation of the baroreflex control of heart rate, probably through a distinct effect on the parasympathetic drive to the heart [39].

It seems that a site-specific action exists for Ang-(1–7) within the brain. For instance, Ang-(1–7) induces similar responses to AngII on BP regulation when it is injected into the RVLM, NTS or CVLM, although these effects are elicited through different mechanisms and receptor subtypes [40,41]. In contrast, Ang-(1–7) exerts opposite actions to those displayed by AngII on baroreflex sensitivity and ischaemic injury [21,29,30]. Up until now, there has been no clear explanation for this differential effect for Ang-(1–7). One may argue that when both peptides act in the same manner, AngII may be metabolized to Ang-(1–7). Despite the fact that both AngII or Ang-(1–7) induced an increase in MAP when injected into the RVLM, several studies have found that blockade of AT_1Rs or AT_2Rs in the RVLM does not alter BP [42,43], but when Mas receptors are blocked a reduction in BP was observed [44,45]. In addition, the pressor effect elicited by AngI into the RVLM was not blocked by an ACE inhibitor, but was reduced by a Mas receptor antagonist, reinforcing the role of endogenous Ang-(1–7) in this area. Another possible explanation may be that Ang-(1–7) binds to AT_1Rs and in this way it elicits responses similar to AngII; however, this hypothesis can be disregarded because the effect of Ang-(1–7) was not modified by an AT_1R antagonist [46]. It seems that the counterbalancing effect of Ang-(1–7) on the actions of AngII depends on the particular cerebral area. Is there something specific to these control centres (NTS, RVLM and CVLM) that could explain this finding? To date, we do not know.

The PVN in the hypothalamus is one of the major sources of afferent inputs to sympathetic pre-ganglionic neurons which control the heart, blood vessels and adrenal medulla. In addition, the PVN also projects to other autonomic nuclei in the brainstem, which may in turn influence the sympathetic vasomotor outflow. Ang-(1–7) has been shown to be as effective as AngII in enhancing cardiac sympathetic afferent reflexes and increasing sympathetic outflow when injected into the PVN of renovascular hypertensive rats [47]. In addition, both endogenous Ang-(1–7) and AngII in the PVN contribute to enhanced cardiac sympathetic afferent reflexes and sympathetic outflow in renovascular hypertension [47]. Furthermore, chronic infusion of both Mas receptor and AT_1R antagonists into the PVN prevents hypertension in a rat model of sleep apnoea [48].

Ang-(1–7) AND NEUROTRANSMITTER RELEASE IN THE BRAIN

Several lines of evidence in animals and humans suggest that sympathetic nervous system overactivity is a primary contributor to the development and maintenance of hypertension. Sympathetic nervous system overactivity may result from either inappropriately elevated sympathetic drive from brain centres, an increase in synaptically released neurotransmitters or amplification of the neurotransmitter signal at the target tissue [49]. The

catecholamines dopamine, noradrenaline (norepinephrine) and adrenaline (epinephrine), acting as neurotransmitters, play important roles in the sympathetic control of BP, both centrally and peripherally [49].

AngII has been shown to exert a stimulatory neuromodulatory action on the brain noradrenaline system [50]. AngII, by activating AT_1Rs, induces an increase in noradrenaline release and synthesis centrally. In contrast, Ang-(1–7) elicits an inhibitory neuromodulatory response in the brain noradrenaline system by acting at three levels: synthesis, release and uptake. Ang-(1–7), acting in sympathetic neurons through Mas receptor or AT_2R stimulation, induces a decrease in neurotransmitter synthesis and release, as well as an increase in noradrenaline uptake, leading to a decrease in neurotransmitter levels in the synaptic cleft [29]. The Ang-(1–7)-stimulated noradrenaline uptake is the result of Ang-(1–7) action solely on neurons, since Mas receptors were shown to be present in neurons and not in astroglia [21], another cell assisting in the termination of signalling molecules that diffuse away from the synapse. In addition, ACE2 was also demonstrated to be present in neurons and not in glial cells [11], reinforcing again the role of Ang-(1–7) in neurons.

With regard to other central neurotransmitters, it has been shown that Ang-(1–7) caused a significant increase in dopamine and GABA (γ-aminobutyric acid) release in the rat stratium, but had no effect on glutamate release [51]. The Ang-(1–7)-induced dopamine release was blocked by an inhibitor of aminopeptidase A, an enzyme which converts Ang-(1–7) into Ang-(3–7), suggesting that this effect occurs after metabolism into Ang-(3–7). In contrast, inhibition of aminopeptidase A had no effect on the Ang-(1–7)-induced GABA release. The Ang-(1–7)-mediated GABA release, but not dopamine release, was blocked by a Mas receptor antagonist, suggesting that only the observed effects on GABA release are mediated by Mas receptors [51].

Thus Ang-(1–7) may contribute to the overall central effects by selectively regulating synaptic neurotransmitter levels.

BEYOND THE BRAIN EFFECTS OF Ang-(1–7) ON BLOOD PRESSURE

Ang-(1–7) not only participates in BP or baroreflex regulation in the brain, but has been shown to improve object recognition memory function [52]. Furthermore, central administration of Ang-(1–7) induces anxiolytic-like effects and decreased oxidative stress in the amygdale of rats [53].

A cerebroprotective action has been described for centrally administered Ang-(1–7) in ischaemic stroke in the endothelin-1-induced middle cerebral artery occlusion model [21]. Ang-(1–7), acting via its Mas receptor, treated rats had reduced cerebral infarct size and improved performance on neurological examinations [21]. These beneficial actions of Ang-(1–7) were due to the attenuation of iNOS [inducible NOS (NO synthase)], pro-inflammatory cytokines and microglia activation [54]. Indeed, the neuroprotective action of Ang-(1–7) in ischaemic stroke has been shown to involve the Mas-receptor-mediated suppression of the inflammatory NF-κB (nuclear factor κB) pathway [55]. Another mediator involved in the protective effect of Ang-(1–7) may be NO. Ang-(1–7) stimulates NO release and up-regulates eNOS (endothelial NOS) expression in ischaemic tissues following focal cerebral ischaemia/reperfusion in rats [56]. The cerebroprotective effect elicited by Ang-(1–7) has also been described in stroke-prone SHRs [57]. Intracerebroventricular infusion of Ang-(1–7) increased the survival time in these rats. Ang-(1–7) treatment also decreased the number of haemorrhages in the striatum, improved neurological status (reduced lethargy), decreased the number of microglia in the striatum and tended to increase neuron survival at the same site [57]. Recently, it has been shown that cerebral infarction resulted in a significant increase in Ang-(1–7) and Mas receptor levels, as well as ACE2 levels, in the cerebral cortex compared with sham-operated rats, reinforcing the concept that this axis plays a pivotal role in the regulation of acute neuron injury in ischaemic cerebrovascular diseases [58].

Chronic treatment with Ang-(1–7) is beneficial in attenuating hypertension-induced pathophysiological changes in the brain. Intracerebroventricular infusion of Ang-(1–7) for 4 weeks significantly reduced iNOS and the NADPH oxidase subunit gp91 in the brain of SHRs [59]. The increase in apoptotic neurons was also attenuated by Ang-(1–7). These antioxidative and anti-apoptotic effects caused by chronic infusion of Ang-(1–7) in SHRs were accompanied by a reduction in the expression of AngII and AT_1Rs, and were independent of BP reduction [59]. Furthermore, Ang-(1–7) was reported to prevent excessive hypertension-induced autophagic activation through Mas receptors and AT_2Rs in brain of SHRs [60]. These studies demonstrate that Ang-(1–7) may be helpful in preventing hypertension-related cerebrovascular diseases [59,60].

Despite the fact that several findings point to a cerebroprective role for Ang-(1–7), it has been reported recently that Mas receptor deficiency induced an increase in the number of young doublecortin-positive neurons in the piriform cortex, an area related to adult neurogenesis [61]. In contrast, deletion of the Mas receptor did not alter cell proliferation in the adult dentatus gyrus, another area capable of adult neurogenesis [61]. This results suggests that blockade of Mas receptors might be beneficial in stimulating neurogenesis in adults [61].

DOWNSTREAM Ang-(1–7) SIGNALLING IN THE BRAIN

In the brain, the interaction of Ang-(1–7) with the Mas receptor has been associated with the activation of several intracellular signalling pathways. NO generation mediated by Mas receptor activation has been demonstrated [56,62–64]. One of the mechanisms by which NO is generated is through the activation of bradykinin B_2 receptors. Ang-(1–7), through Mas receptor stimulation, induces bradykinin generation which, in turn, activates B_2 receptors, with the subsequent activation of NOS and thus NO generation [64]. In differentiated catecholaminergic neurons, Ang-(1–7) is capable of increasing nNOS (neuronal NOS)-derived NO levels, which activate the hyperpolarizing voltage-gated outward K^+ current [65].

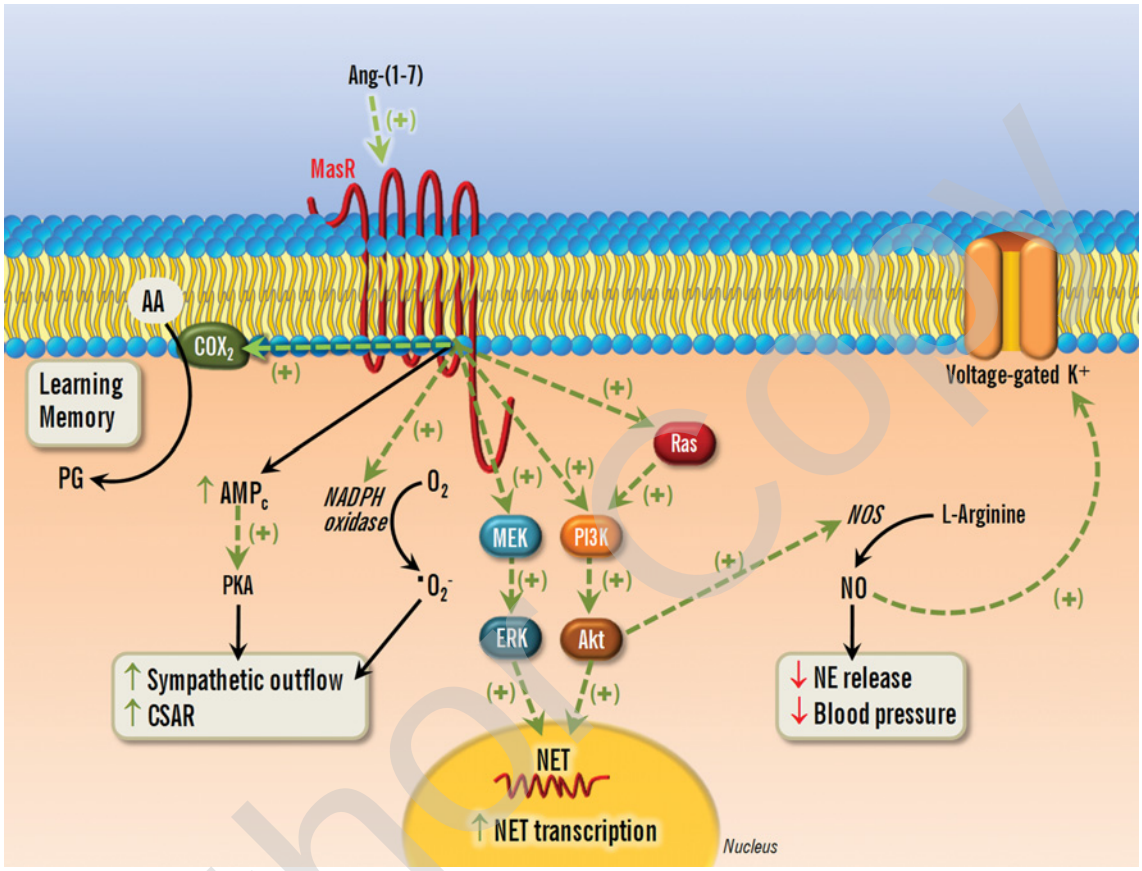

Figure 3 **Proposed Ang-(1–7) signalling in the central nervous system**
Abbreviations: AA, arachidonic acid; CSAR, cardiac sympathetic afferent reflex; Ikv, voltage-gated outward K^+; MasR, Mas receptor; NET, noradrenaline transporter; NE, noradrenaline; PG, prostaglandin.

In neurons from the hypothalamus, Mas receptor stimulation by Ang-(1–7) is coupled with PI3K (phosphoinositide 3-kinase)/Akt- and MEK1/2 (MAPK/ERK kinase 1/2)/ERK1/2 (extracellular-signal-regulated kinase 1/2)-dependent signalling pathways, and therefore Ang-(1–7) induces changes in the gene transcription of the noradrenaline transporter [22]. Another mediator of the actions of Ang-(1–7) is COX2 (cyclooxygenase 2), the key enzyme that converts arachidonic acid into prostaglandins, as it has been shown that Ang-(1–7)-induced plasticity changes in the lateral amygdala occur via COX-2 [62].

Activation of Mas receptors by Ang-(1–7) in the PVN or RVLM of renovascular hypertensive rats is associated with the cAMP/PKA (protein kinase A) pathway, which mediates the enhanced sympathetic outflow and cardiac sympathetic afferent reflex elicited by the heptapeptide [66,67]. In this context, it has been demonstrated that superoxide anions are the signalling molecules implicated in the sympatho-excitatory effect of Ang-(1–7) mediated by the Mas receptor in the RVLM. NADPH oxidase is the major source of the superoxide anions that modulate the effects of Ang-(1–7) in the RVLM [68]; however, the mechanism of NADPH oxidase activation by Ang-(1–7) in the RVLM is still not well understood.

Interestingly, it has been shown that renin may mediate the central control of BP at the NTS through the Ras/PI3K/Akt signalling pathway to regulate the phosphorylation of eNOS. In this way, renin modulates BP via centrally located AT_1Rs and Mas receptors, which activate Ras, PI3K, Akt and eNOS phosphorylation [69].

Figure 3 summarizes the signalling pathways coupled to the Ang-(1–7)/Mas receptor axis.

ACE2 IN THE BRAIN

In the brain, ACE2 is expressed in neurons [11] and astroglial cells [12]. ACE2 is an integral membrane protein with its catalytic site exposed to circulating vasoactive peptides [70]. In a human liver cell line endogenously expressing ACE2, it has been shown that ACE2 attachment to the cell membrane is regulated by calmodulin binding through a calcium-dependent calmodulin–peptide complex involving the ACE2 cytoplasmic domain [71,72]. The enzyme is released from the plasma membrane in response to phorbol ester stimuli by TNF (tumour necrosis factor)-α-converting enzyme (ADAM17), a disintegrin and metalloproteinase, resulting in the formation of a soluble

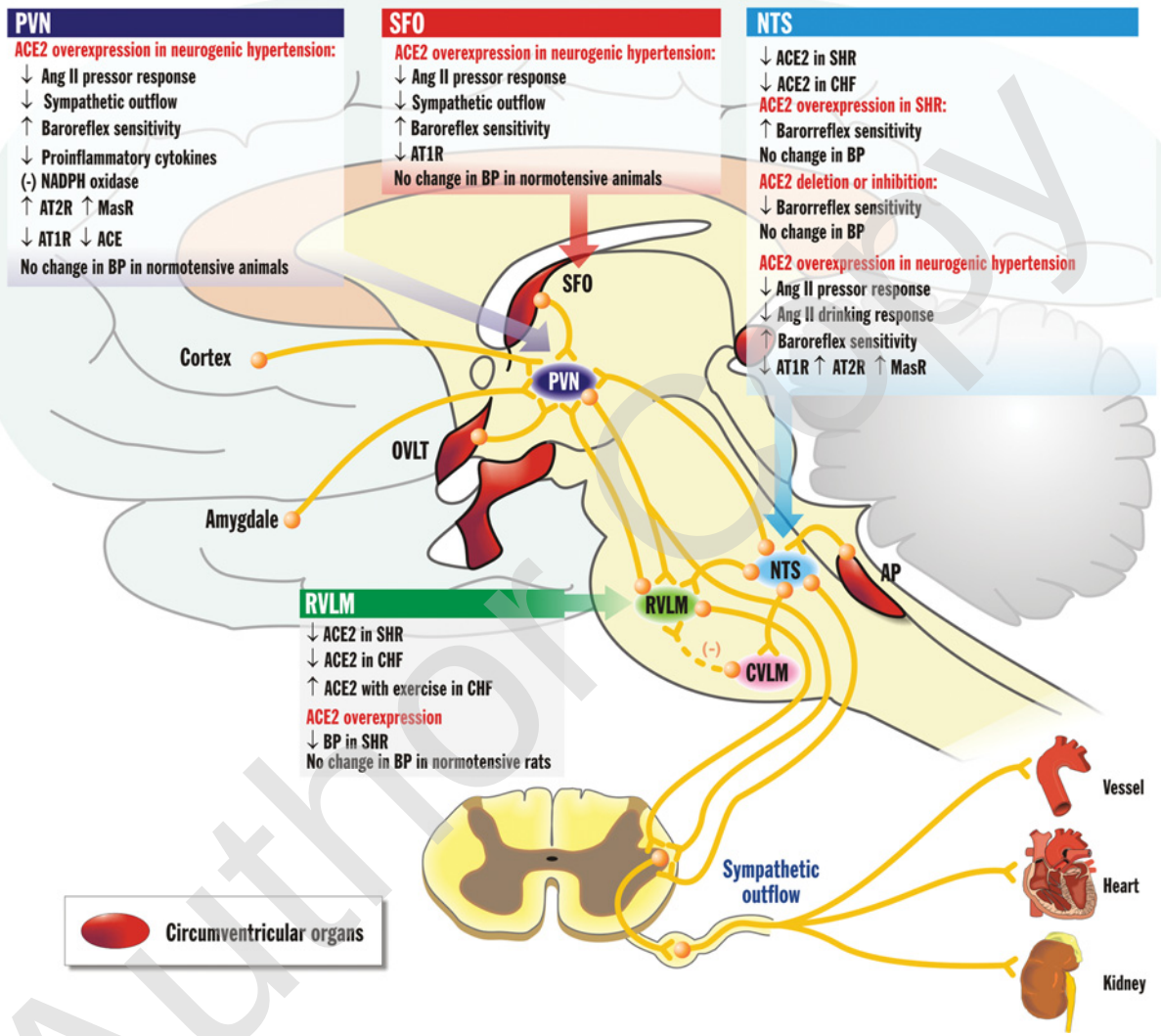

Figure 4 **ACE2 at the central level**
Abbreviations: AP, anterior hypothalamus; MasR, Mas receptor.

truncated, but active, ACE2 [sACE2 (soluble ACE2)] [71–73]. This process is called ACE2 shedding [71–73]. sACE2 has been also observed in the cytoplasm of neurons in the mouse brain, probably due to endocytosis of the secreted enzyme [11]. Modulation of ACE2 membrane attachment may be of physiological and pathophysiological importance, as it determinates the levels of ACE2 as an integral membrane protein, the levels of circulatory sACE2 and the levels of intracellular sACE2. Recently, it has been shown that ADAM17 levels were up-regulated in DOCA (deoxycorticosterone acetate)-salt-induced hypertension, which contributes to ACE2 shedding [74]. The knockdown of ADAM17 prevented the reduction in ACE2 levels in the brain and blunted DOCA salt-induced hypertension, showing that ACE2 shedding contributes to the development of neurogenic hypertension [74].

AngI and AngII are not the only substrates for ACE2. Other substrates of physiological relevance are apelin, particularly apelin-13 and apelin-36 [10], and AngA [75,76]. ACE2 hydrolyses apelin or AngII with similar catalytic efficiency [77]. Intracerebroventricular administration of apelin does not modify BP [78], but its administration into the SFO decreases BP and heart rate as a consequence of its modulating effects on neuron excitability in this area [79]. A neuroprotective role has been attributed to apelin, as it has been shown that it attenuates neuron apoptosis after ischaemia/reperfusion injury [80]. ACE2 also cleaves AngA, which exerts pressor actions [76] to yield the recently described heptapeptide alamandine. The novel peptide alamandine elicits biological activities that resemble those of Ang-(1–7) [75].

ACE2 expression is decreased in hypertensive rat strains [81]. Yamazato et al. [82,83] showed that ACE2 expression is reduced in the RVLM and NTS of SHRs compared with Wistar–Kyoto rats. In agreement, ACE2 expression has also been reported to be reduced in neurogenic hypertension [84] and in CHF (chronic heart failure) [85,86], another important cardiovascular pathology. In contrast, exercise induced an increase in central ACE2

expression, thus reversing the imbalance of ACE2 in regions of the brain that regulate autonomic function [85]. Given the fact that ACE2 expression is decreased in SHRs, overexpression of murine ACE2 in the RVLM of SHRs and Wistar–Kyoto rats succeeded in lowering BP in the hypertensive strain, probably because this gene therapy ensured that SHRs overcame the ACE2 deficiency [82]. In contrast, overexpression of murine ACE2 in the NTS failed to decrease BP but increased baroreflex sensitivity [83].

In neurogenic hypertension, the brain-targeted expression of human ACE2 under the control of a neuron-specific promoter in the SFO or in the PVN attenuated the AngII pressor response and improved autonomic function through augmentation of baroreflex sensitivity and a reduction in sympathetic outflow [87,88]. In mice with CHF, brain-selective overexpression of human ACE2 attenuated CHF and induced a decrease in BP [86].

Interestingly, human ACE2 overexpression in the SFO [87] and in the PVN [88] did not affect BP in control normotensive animals, but attenuated neurogenic hypertension, suggesting that ACE2 plays a role in pathophysiological situations. The fact that murine ACE2 overexpression in the RVLM decreased BP in SHRs but had no effect on Wistar–Kyoto rats [82] reinforces this hypothesis.

Several studies have tried to elucidate the mechanism by which ACE2 overexpression therapy improves neurogenic hypertension. Neuron-selective expression of human ACE2 in the brain exhibited a protective response to AngII pressor stimuli that was reversed by chronic systemic blockade of Mas receptors, suggesting that attenuation of neurogenic hypertension by neuronal human ACE2 overexpression was mediated by the Ang-(1–7)/Mas receptor axis [84]. In addition, up-regulation of NOS expression correlating with augmented NO release was also observed in this model [84]. Sriramula et al. [88] have reported that the attenuation of neurogenic hypertension induced by human ACE2 overexpression in the PVN was accompanied by the up-regulation of AT_2Rs and Mas receptors as well as the down-regulation AT_1Rs and ACE, shifting the RAS to its depressor arm. In addition, AngII-stimulated pro-inflammatory cytokine release was also attenuated [88]. In $ACE2^{-/y}$ mice treated chronically with AngII infusion, human ACE2 overexpression in the PVN induced a decrease in ROS (reactive oxygen species) and AT_1Rs and improved autonomic function [89]. Taken together, these results suggest that central gene-therapy-protective effects of ACE2 on neurogenic hypertension are closely linked to the Ang-(1–7)/Mas receptor pathway.

Regarding CHF, human ACE2 overexpression in the brains of mice attenuated CHF by decreasing sympathetic outflow, and this effect was attenuated by chronic systemic blockade of Mas receptors, suggesting that inhibition of sympathetic activity in mice with CHF by human ACE2 overexpression is mediated by the Ang-(1–7)/Mas receptor axis [86]. In addition, decreased AT_1R levels were also observed in the NTS, but not in the RVLM of mice with CHF [86].

Many studies have highlighted the role of central ACE2 in baroreflex function. Indeed it has been demonstrated that ACE2 deficiency in the NTS from SHRs may be responsible for the impaired baroreflex function observed in this strain [83]. Accordingly, complete ACE2 gene deletion in mice [89] or local ACE2 inhibition in the NTS of rats [90] resulted in reduced baroreflex function. These findings support the concept that, within the NTS, local synthesis of Ang-(1–7) from AngII is required for normal sensitivity for the baroreflex control of heart rate in response to increases in arterial pressure [90].

ACE2 expression and AT_1R levels seem to be intimately connected. Systemic infusion or intracerebroventricular injection of AngII caused ACE2 down-regulation and AT_1R up-regulation in the brain stem, SFO and PVN, which was reversed by human ACE2 overexpression [84,87,88]. Accordingly, AngII stimulation caused a decrease in ACE2 mRNA and protein expression in primary cultures of cerebellar and medullar astrocytes, and this was prevented by AT_1R blockade [12]. ACE2 overexpression not only led to AT_1R down-regulation in the NTS, SFO and PVN, but also increased AT_2R and Mas receptor expression [84,87,88]. Additionally, ACE2 overexpression in the PVN attenuated the AngII-induced increase in the expression of the pro-inflammatory cytokines TNF-α, IL (interleukin)-1β and IL-6 [88]. Taken together, these data suggest that ACE2 overexpression confers a protective effect by modulating angiotensin receptor expression in the brainstem [84,87,88]. Figure 4 summarizes the principal findings concerning ACE2 expression at the central level.

SOME CONSIDERATIONS

It seems that there is a reluctance to determine peptide concentrations and brain distribution, and this may be due to methodological limitations. In addition, all of the studies reporting the effects of Ang-(1–7) employed quantities of the peptide several orders of magnitude higher than those present in the brain. If physiological concentrations are employed, no net effect may be observed, because of the many mechanisms being employed at the same time to maintain the physiological state. Despite the fact that pharmacological concentrations are used to investigate an effect of the peptide, some studies corroborate these results by using gene-deleted mice (where it is possible) or through receptor blockers or antibodies that inhibit the action of the endogenous peptide. However, this last point is limited by the lack of a commercially available antibody against Ang-(1–7). Furthermore, there is some doubt about the effectiveness and specificity of many of the antibodies used. Therefore, where it is possible and to support the findings, data obtained using high concentrations of peptide should be corroborated by other approaches, including gene-deleted mice, specific receptors blockers or specific antibodies that block the action of endogenous hormones or even through inhibition of enzymes involved in hormone generation. In this way, it is possible to confirm the endogenous role of the peptide.

CONCLUSIONS

Our understanding of the RAS has changed considerably from when it was first identified. Originally, it was thought that AngII was the only bioactive component, but this is now not the case

following the identification of other components of the system, including AngIII, AngIV, Ang-(1–7), Ang-(1–12), AngA and, more recently, alamandine [75,76]. These peptides have been described to contribute to the overall actions of the RAS, with some of them favouring an increase in BP, whereas others result in a decrease in BP. The Ang-(1–7)/Mas receptor axis contributes to BP regulation centrally and exerts a cerebroprotective action. In view of its renal- and cardiovascular-protective effects, this axis should be considered as a potential therapeutic target by potentiating its activity. Further investigations into the molecular events underlying the actions of Ang-(1–7) and the regulation of Mas receptors will be required before this becomes a reality.

REFERENCES

1 McKinley, M. J., Albiston, A. L., Allen, A. M., Mathai, M. L., May, C. N., McAllen, R. M., Oldfield, B. J., Mendelsohn, F. A. O. and Chai, S. Y. (2003) The brain renin–angiotensin system: location and physiological roles. Int. J. Biochem. Cell Biol. **35**, 901–918 CrossRef PubMed

2 Marc, Y. and Llorens-Cortes, C. (2011) The role of the brain renin–angiotensin system in hypertension: implications for new treatment. Prog. Neurobiol. **95**, 89–103 CrossRef PubMed

3 Grobe, J. L., Xu, D. and Sigmund, C. D. (2008) An intracellular renin–angiotensin system in neurons: fact, hypothesis, or fantasy. Physiology **23**, 187–293 CrossRef PubMed

4 Kumar, R., Thomas, C. M., Yong, Q. C., Chen, W. and Baker, K. M. (2012) The intracrine renin–angiotensin system. Clin. Sci. **123**, 273–284 CrossRef PubMed

5 Welches, W. R., Santos, R. A., Chappell, M. C., Brosnihan, K. B., Greene, L. J. and Ferrario, C. M. (1991) Evidence that prolyl endopeptidase participates in the processing of brain angiotensin. J. Hypertens. **9**, 631–638 CrossRef PubMed

6 Yamamoto, K., Chappell, M. C., Brosnihan, K. B. and Ferrario, C. M. (1992) *In vivo* metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. Hypertension **19**, 692–696 CrossRef PubMed

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

8 Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., Donovan, M., Woolf, B., Robison, K., Jeyaseelan, R. et al. (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ. Res. **87**, E1–E9 CrossRef PubMed

9 Tipnis, S. R., Hooper, N. M., Hyde, R., Karran, E., Christie, G. and Turner, A. J. (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J. Biol. Chem. **275**, 33238–33243 CrossRef PubMed

10 Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J., Godbout, K., Parsons, T., Baronas, E., Hsieh, F. et al. (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J. Biol. Chem. **277**, 14838–14843 CrossRef PubMed

11 Doobay, M. F., Talman, L. S., Obr, T. D., Tian, X., Davisson, R. L. and Lazartigues, E. (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin–angiotensin system. Am. J. Physiol. Regul. Integr. Comp. Physiol. **292**, R373–R381 CrossRef PubMed

12 Gallagher, P. E., Chappell, M. C., Ferrario, C. M. and Tallant, E. A. (2006) Distinct roles for ANG II and ANG-(1–7) in the regulation of angiotensin-converting enzyme 2 in rat astrocytes. Am. J. Physiol. Cell Physiol. **290**, C420–C426 CrossRef PubMed

13 Block, C. H., Santos, R. A., Brosnihan, K. B. and Ferrario, C. M. (1988) Immunocytochemical localization of angiotensin-(1–7) in the rat forebrain. Peptides **9**, 1395–1401 CrossRef PubMed

14 Lopez Verrilli, M. A., Pirola, C. J., Pascual, M. M., Dominici, F. P., Turyn, D. and Gironacci, M. M. (2009) Angiotensin-(1–7) through AT2 receptors mediates tyrosine hydroxylase degradation via the ubiquitin-proteasome pathway. J. Neurochem. **109**, 326–335 CrossRef PubMed

15 Arnold, A. C., Sakima, A., Kasper, S. O., Vinsant, S., Garcia-Espinosa, M. A. and Diz, D. I. (2012) The brain renin–angiotensin system and cardiovascular responses to stress: insights from transgenic rats with low brain angiotensinogen. J. Appl. Physiol. **113**, 1929–1936 CrossRef PubMed

16 Chappell, M. C., Brosnihan, K. B., Diz, D. I. and Ferrario, C. M. (1989) Identification of angiotensin-(1–7) in rat brain. Evidence for differential processing of angiotensin peptides. J. Biol. Chem. **264**, 16518–16523 PubMed

17 Pereira, M. G., Souza, L. L., Becari, C., Duarte, D. A., Camacho, F. R., Oliveira, J. A., Gomes, M. D., Oliveira, E. B., Salgado, M. C., Garcia-Cairasco, N. and Costa-Neto, C. M. (2013) Angiotensin II-independent angiotensin-(1–7) formation in rat hippocampus: involvement of thimet oligopeptidase. Hypertension **62**, 879–885 CrossRef PubMed

18 Gouveia, T. L., Frangiotti, M. I., de Brito, J. M., de Castro Neto, E. F., Sakata, M. M., Febba, A. C., Casarini, D. E., Amado, D., Cavalheiro, E. A., Almeida, S. S. et al. (2012) The levels of renin–angiotensin related components are modified in the hippocampus of rats submitted to pilocarpine model of epilepsy. Neurochem. Int. **61**, 54–62 CrossRef PubMed

19 Santos, R. A., Simoes e Silva, A. C., Maric, C., Silva, D. M., Machado, R. P., de Buhr, I., Heringer-Walther, S., Pinheiro, S. V., Lopes, M. T., Bader, M. 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 CrossRef PubMed

20 Becker, L. K., Etelvino, G. M., Walther, T., Santos, R. A. and Campagnole-Santos, M. J. (2007) Immunofluorescence localization of the receptor Mas in cardiovascular-related areas of the rat brain. Am. J. Physiol. Heart Circ. Physiol. **293**, H1416–H1424 CrossRef PubMed

21 Mecca, A. P., Regenhardt, R. W., O'Connor, T. E., Joseph, J. P., Raizada, M. K., Katovich, M. J. and Sumners, C. (2011) Cerebroprotection by angiotensin-(1–7) in endothelin-1-induced ischaemic stroke. Exp. Physiol. **96**, 1084–1096 PubMed

22 Lopez Verrilli, M. A., Rodriguez Fermepin, M., Longo Carbajosa, N., Landa, S., Cerrato, B. D., Garcia, S., Fernandez, B. E. and Gironacci, M. M. (2012) Angiotensin-(1–7) through Mas receptor up-regulates neuronal norepinephrine transporter via Akt and Erk1/2-dependent pathways. J. Neurochem. **120**, 46–55 CrossRef PubMed

23 Freund, M., Walther, T. and von Bohlen und Halbach, O. (2012) Immunohistochemical localization of the angiotensin-(1–7) receptor Mas in the murine forebrain. Cell Tissue Res. **348**, 29–35 CrossRef PubMed

24 Campagnole-Santos, M. J., Diz, D. I., Santos, R. A., Khosla, M. C., Brosnihan, K. B. and Ferrario, C. M. (1989) Cardiovascular effects of angiotensin-(1–7) injected into the dorsal medulla of rats. Am. J. Physiol. **257**, H324–H329 PubMed

25 Dampney, R. A. (1994) Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. **74**, 323–364 PubMed

26 Dampney, R. A., Horiuchi, J., Killinger, S., Sheriff, M. J., Tan, P. S. and McDowall, L. M. (2005) Long-term regulation of arterial blood pressure by hypothalamic nuclei: some critical questions. Clin. Exp. Pharmacol. Physiol. **32**, 419–425 CrossRef PubMed

27 Couto, A. S., Baltatu, O., Santos, R. A., Ganten, D., Bader, M. and Campagnole-Santos, M. J. (2002) Differential effects of angiotensin II and angiotensin-(1–7) at the nucleus tractus solitarii of transgenic rats with low brain angiotensinogen. J. Hypertens. **20**, 919–925 CrossRef PubMed

28 Thrasher, T. N. (2004) Baroreceptors and the long-term control of blood pressure. Exp. Physiol. **89**, 331–335 CrossRef PubMed

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

30 Oliveira, D. R., Santos, R. A., Santos, G. F., Khosla, M. and Campagnole-Santos, M. J. (1996) Changes in the baroreflex control of heart rate produced by central infusion of selective angiotensin antagonists in hypertensive rats. Hypertension **27**, 1284–1290 CrossRef PubMed

31 Arnold, A. C., Sakima, A., Ganten, D., Ferrario, C. M. and Diz, D. I. (2008) Modulation of reflex function by endogenous angiotensins in older transgenic rats with low glial angiotensinogen. Hypertension **51**, 1326–1331 CrossRef PubMed

32 Diz, D. I., Garcia-Espinosa, M. A., Gallagher, P. E., Ganten, D., Ferrario, C. M. and Averill, D. B. (2008) Angiotensin-(1–7) and baroreflex function in nucleus tractus solitarii of (mRen2)27 transgenic rats. J. Cardiovasc. Pharmacol. **51**, 542–548 CrossRef PubMed

33 Nautiyal, M., Shaltout, H. A., de Lima, D. C., do Nascimento, K., Chappell, M. C. and Diz, D. I. (2012) Central angiotensin-(1–7) improves vagal function independent of blood pressure in hypertensive (mRen2)27 rats. Hypertension **60**, 1257–1265 CrossRef PubMed

34 Garcia-Espinosa, M. A., Shaltout, H. A., Gallagher, P. E., Chappell, M. C. and Diz, D. I. (2012) *In vivo* expression of angiotensin-(1–7) lowers blood pressure and improves baroreflex function in transgenic (mRen2)27 rats. J. Cardiovasc. Pharmacol. **60**, 150–157 CrossRef PubMed

35 Isa, K., Arnold, A. C., Westwood, B. M., Chappell, M. C. and Diz, D. I. (2011) Angiotensin-converting enzyme inhibition, but not AT_1 receptor blockade, in the solitary tract nucleus improves baroreflex sensitivity in anesthetized transgenic hypertensive (mRen2)27 rats. Hypertens. Res. **34**, 1257–1262 CrossRef PubMed

36 de Moura, M. M., dos Santos, R. A., Campagnole-Santos, M. J., Todiras, M., Bader, M., Alenina, N. and Haibara, A. S. (2010) Altered cardiovascular reflexes responses in conscious angiotensin-(1–7) receptor Mas-knockout mice. Peptides **31**, 1934–1939 CrossRef PubMed

37 Shaltout, H. A., Rose, J. C., Chappell, M. C. and Diz, D. I. (2012) Angiotensin-(1–7) deficiency and baroreflex impairment precede the antenatal Betamethasone exposure-induced elevation in blood pressure. Hypertension **59**, 453–458 CrossRef PubMed

38 Marshall, A. C., Shaltout, H. A., Nautiyal, M., Rose, J. C., Chappell, M. C. and Diz, D. I. (2013) Fetal betamethasone exposure attenuates angiotensin-(1–7)-Mas receptor expression in the dorsal medulla of adult sheep. Peptides **44**, 25–31 CrossRef PubMed

39 Alzamora, A. C., Santos, R. A. and Campagnole-Santos, M. J. (2006) Baroreflex modulation by angiotensins at the rat rostral and caudal ventrolateral medulla. Am. J. Physiol. Regul. Integr. Comp. Physiol. **290**, R1027–R1034 CrossRef PubMed

40 Ferreira, P. M., Alzamora, A. C., Santos, R. A. and Campagnole-Santos, M. J. (2008) Hemodynamic effect produced by microinjection of angiotensins at the caudal ventrolateral medulla of spontaneously hypertensive rats. Neuroscience **151**, 1208–1216 CrossRef PubMed

41 Alzamora, A. C., Santos, R. A. and Campagnole-Santos, M. J. (2002) Hypotensive effect of ANG II and ANG-(1–7) at the caudal ventrolateral medulla involves different mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. **283**, R1187–R1195 PubMed

42 Hirooka, Y., Potts, P. D. and Dampney, R. A. (1997) Role of angiotensin II receptor subtypes in mediating the sympathoexcitatory effects of exogenous and endogenous angiotensin peptides in the rostral ventrolateral medulla of the rabbit. Brain Res. **772**, 107–114 CrossRef PubMed

43 Lin, K. S., Chan, J. Y. and Chan, S. H. (1997) Involvement of AT2 receptors at NRVL in tonic baroreflex suppression by endogenous angiotensins. Am. J. Physiol. **272**, H2204–H2210 PubMed

44 Fontes, M. A., Martins Pinge, M. C., Naves, V., Campagnole-Santos, M. J., Lopes, O. U., Khosla, M. C. and Santos, R. A. (1997) Cardiovascular effects produced by microinjection of angiotensins and angiotensin antagonists into the ventrolateral medulla of freely moving rats. Brain Res. **750**, 305–310 CrossRef PubMed

45 Lima, D. X., Fontes, M. A., Oliveira, R. C., Campagnole-Santos, M. J., Khosla, M. C. and Santos, R. A. (1996) Pressor action of angiotensin I at the ventrolateral medulla: effect of selective angiotensinblockade. Immunopharmacology **33**, 305–307 CrossRef PubMed

46 Fontes, M. A., Silva, L. C., Campagnole-Santos, M. J., Khosla, M. C., Guertzenstein, P. G. and Santos, R. A. (1994) Evidence that angiotensin-(1–7) plays a role in the central control of blood pressure at the ventro-lateral medulla acting through specific receptors. Brain Res. **665**, 175–180 CrossRef PubMed

47 Sun, H. J., Li, P., Chen, W. W., Xiong, X. Q. and Han, Y. (2012) Angiotensin II and angiotensin-(1–7) in paraventricular nucleus modulate cardiac sympathetic afferent reflex in renovascular hypertensive rats. PLoS ONE **7**, e52557 CrossRef PubMed

48 da Silva, A. Q., Fontes, M. A. and Kanagy, N. L. (2011) Chronic infusion of angiotensin receptor antagonists in the hypothalamic paraventricular nucleus prevents hypertension in a rat model of sleep apnea. Brain Res. **1368**, 231–238 CrossRef PubMed

49 Wyss, J. M. and Carlson, S. H. (2001) The role of the nervous system in hypertension. Curr. Hypert. Rep. **3**, 255–262 CrossRef

50 Gelband, C. H., Sumners, C. and Raizada, M. K. (1997) Angiotensin receptors and norepinephrine neuromodulation: implications of functional coupling. Regul. Pept. **72**, 139–145 CrossRef PubMed

51 Stragier, B., Hristova, I., Sarre, S., Ebinger, G. and Michotte, Y. (2005) *In vivo* characterization of the angiotensin-(1–7)-induced dopamine and gamma-aminobutyric acid release in the striatum of the rat. Eur. J. Neurosci. **22**, 658–664 CrossRef PubMed

52 Lazaroni, T. L., Raslan, A. C., Fontes, W. R., de Oliveira, M. L., Bader, M., Alenina, N., Moraes, M. F., Dos Santos, R. A. and Pereira, G. S. (2012) Angiotensin-(1–7)/Mas axis integrity is required for the expression of object recognition memory. Neurobiol. Learn. Mem. **97**, 113–123 CrossRef PubMed

53 Bild, W. and Ciobica, A. (2013) Angiotensin-(1–7) central administration induces anxiolytic-like effects in elevated plus maze and decreased oxidative stress in the amygdala. J. Affect. Disord. **145**, 165–171 CrossRef PubMed

54 Regenhardt, R. W., Desland, F., Mecca, A. P., Pioquinto, D. J., Afzal, A., Mocco, J. and Sumners, C. (2013) Anti-inflammatory effects of angiotensin-(1–7) in ischemic stroke. Neuropharmacology **71**, 154–63 CrossRef PubMed

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

56 Zhang, Y., Lu, J., Shi, J., Lin, X., Dong, J., Zhang, S., Liu, Y. and Tong, Q. (2008) Central administration of angiotensin-(1–7) stimulates nitric oxide release and upregulates the endothelial nitric oxide synthase expression following focal cerebral ischemia/reperfusion in rats. Neuropeptides **42**, 593–600 CrossRef PubMed

57 Regenhardt, R. W., Mecca, A. P., Desland, F., Ritucci-Chinni, P. F., Ludin, J. A., Greenstein, D., Banuelos, C., Bizon, J. L., Reinhard, M. K. and Sumners, C. (2014) Centrally administered angiotensin-(1–7) increases the survival of stroke-prone spontaneously hypertensive rats. Exp Physiol. **99**, 442–453 CrossRef PubMed

58 Lu, J., Jiang, T., Wu, L., Gao, L., Wang, Y., Zhou, F., Zhang, S. and Zhang, Y. (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 CrossRef PubMed

59 Jiang, T., Gao, L., Shi, J., Lu, J., Wang, Y. and Zhang, Y. (2013) Angiotensin-(1–7) modulates renin–angiotensin system associated with reducing oxidative stress and attenuating neuronal apoptosis in the brain of hypertensive rats. Pharmacol. Res. **67**, 84–89 CrossRef PubMed

60 Jiang, T., Gao, L., Zhu, X. C., Yu, J. T., Shi, J. Q., Tan, M. S., Lu, J., Tan, L. and Zhang, Y. D. (2013) Angiotensin-(1–7) inhibits autophagy in the brain of spontaneously hypertensive rats. Pharmacol. Res. **71**, 61–68 CrossRef PubMed

61 Freund, M., Walther, T. and von Bohlen Und Halbach, O. (2013) 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 CrossRef PubMed

62 Albrecht, D. (2007) Angiotensin-(1–7)-induced plasticity changes in the lateral amygdala are mediated by COX-2 and NO. Learn. Mem. **14**, 177–184 CrossRef PubMed

63 Xing, J., Kong, J., Lu, J. and Li, J. (2012) Angiotensin-(1–7) inhibits neuronal activity of dorsolateral periaqueductal gray via a nitric oxide pathway. Neurosci. Lett. **522**, 156–161 CrossRef PubMed

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

65 Yang, R. F., Yin, J. X., Li, Y. L., Zimmerman, M. C. and Schultz, H. D. (2011) Angiotensin-(1–7) increases neuronal potassium current via a nitric oxide-dependent mechanism. Am. J. Physiol. Cell. Physiol. **300**, C58–C64 CrossRef PubMed

66 Li, P., Sun, H. J., Cui, B. P., Zhou, Y. B. and Han, Y. (2013) Angiotensin-(1–7) in the rostral ventrolateral medulla modulates enhanced cardiac sympathetic afferent reflex and sympathetic activation in renovascular hypertensive rats. Hypertension **61**, 820–827 CrossRef PubMed

67 Han, Y., Sun, H. J., Li, P., Gao, Q., Zhou, Y. B., Zhang, F., Gao, X. Y. and Zhu, G. Q. (2012) Angiotensin-(1–7) in paraventricular nucleus modulates sympathetic activity and cardiac sympathetic afferent reflex in renovascular hypertensive rats. PLoS ONE **7**, e48966 CrossRef PubMed

68 Li, P., Zhang, F., Zhou, Y. B., Cui, B. P. and Han, Y. (2012) Superoxide anions modulate the effects of angiotensin-(1–7) in the rostral ventrolateral medulla on cardiac sympathetic afferent reflex and sympathetic activity in rats. Neuroscience **223**, 388–398 CrossRef PubMed

69 Cheng, W. H., Lu, P. J., Hsiao, M., Hsiao, C. H., Ho, W. Y., Cheng, P. W., Lin, C. T., Hong, L. Z. and Tseng, C. J. (2012) Renin activates PI3K-Akt-eNOS signalling through the angiotensin AT_1 and Mas receptors to modulate central blood pressure control in the nucleus tractus solitarii. Br. J. Pharmacol. **166**, 2024–2035 CrossRef PubMed

70 Clarke, N. E. and Turner, A. J. (2012) Angiotensin-converting enzyme 2: the first decade. Int. J. Hypertens. **2012**, 307–315 CrossRef

71 Lambert, D. W., Clarke, N. E., Hooper, N. M. and Turner, A. J. (2008) Calmodulin interacts with angiotensin-converting enzyme-2 (ACE2) and inhibits shedding of its ectodomain. FEBS Lett. **582**, 385–390 CrossRef PubMed

72 Lai, Z. W., Lew, R. A., Yarski, M. A., Mu, F. T., Andrews, R. K. and Smith, A. I. (2009) The identification of a calmodulin-binding domain within the cytoplasmic tail of angiotensin-converting enzyme-2. Endocrinology **150**, 2376–2381 CrossRef PubMed

73 Lambert, D. W., Yarski, M., Warner, F. J., Thornhill, P., Parkin, E. T., Smith, A. I., Hooper, N. M. and Turner, A. J. (2005) Tumor necrosis factor-α convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). J. Biol. Chem. **280**, 30113–30119 CrossRef PubMed

74 Xia, H., Sriramula, S., Chhabra, K. H. and Lazartigues, E. (2013) Brain angiotensin-converting enzyme type 2 shedding contributes to the development of neurogenic hypertension. Circ. Res. **113**, 1087–1096 CrossRef PubMed

75 Lautner, R. Q., Villela, D. C., Fraga-Silva, R. A., Silva, N., Verano-Braga, T., Costa-Fraga, F., Jankowski, J., Jankowski, V., Sousa, F., Alzamora, A. et al. (2013) Discovery and characterization of alamandine: a novel component of the renin–angiotensin system. Circ. Res. **112**, 1104–1111 CrossRef PubMed

76 Carey, R. M. (2013) Newly discovered components and actions of the renin–angiotensin system. Hypertension **62**, 818–822 CrossRef PubMed

77 Kazemi-Bajestani, S. M., Patel, V. B., Wang, W. and Oudit, G. Y. (2012) Targeting the ACE2 and apelin pathways are novel therapies for heart failure: opportunities and challenges. Cardiol. Res. Pract. **2012**, 823193 PubMed

78 Falcao-Pires, I. and Leite-Moreira, A. F. (2005) Apelin: a novel neurohumoral modulator of the cardiovascular system. Pathophysiologic importance and potential use as a therapeutic target. Rev. Port. Cardiol. **24**, 1263–1276 PubMed

79 Dai, L., Smith, P. M., Kuksis, M. and Ferguson, A. V. (2013) Apelin acts in the subfornical organ to influence neuronal excitability and cardiovascular function. J. Physiol. **591**, 3421–3432 CrossRef PubMed

80 Khaksari, M., Aboutaleb, N., Nasirinezhad, F., Vakili, A. and Madjd, Z. (2012) Apelin-13 protects the brain against ischemic reperfusion injury and cerebral edema in a transient model of focal cerebral ischemia. J. Mol. Neurosci. **48**, 201–208 CrossRef PubMed

81 Crackower, M. A., Sarao, R., Oudit, G. Y., Yagil, C., Kozieradzki, I., Scanga, S. E., Oliveira-dos-Santos, A. J., da Costa, J., Zhang, L., Pei, Y. et al. (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature **417**, 822–828 CrossRef PubMed

82 Yamazato, M., Yamazato, Y., Sun, C., Diez-Freire, C. and Raizada, M. K. (2007) Overexpression of angiotensin-converting enzyme 2 in the rostral ventrolateral medulla causes long-term decrease in blood pressure in the spontaneously hypertensive rats. Hypertension **49**, 926–931 CrossRef PubMed

83 Yamazato, M., Ferreira, A. J., Yamazato, Y., Diez-Freire, C., Yuan, L., Gillies, R. and Raizada, M. K. (2011) Gene transfer of angiotensin-converting enzyme 2 in the nucleus tractus solitarius improves baroreceptor heart rate reflex in spontaneously hypertensive rats. J. Renin Angiotensin Aldosterone Syst. **12**, 456–461 CrossRef PubMed

84 Feng, Y., Xia, H., Cai, Y., Halabi, C. M., Becker, L. K., Santos, R. A., Speth, R. C., Sigmund, C. D. and Lazartigues, E. (2010) Brain-selective overexpression of human Angiotensin-converting enzyme type 2 attenuates neurogenic hypertension. Circ. Res. **106**, 373–382 CrossRef PubMed

85 Kar, S., Gao, L. and Zucker, I. H. (2010) Exercise training normalizes ACE and ACE2 in the brain of rabbits with pacing-induced heart failure. J. Appl. Physiol. **108**, 923–932 CrossRef PubMed

86 Xiao, L., Gao, L., Lazartigues, E. and Zucker, I. H. (2011) Brain-selective overexpression of angiotensin-converting enzyme 2 attenuates sympathetic nerve activity and enhances baroreflex function in chronic heart failure. Hypertension **58**, 1057–1065 CrossRef PubMed

87 Feng, Y., Yue, X., Xia, H., Bindom, S. M., Hickman, P. J., Filipeanu, C. M., Wu, G. and Lazartigues, E. (2008) Angiotensin-converting enzyme 2 overexpression in the subfornical organ prevents the angiotensin II-mediated pressor and drinking responses and is associated with angiotensin II type 1 receptor downregulation. Circ. Res. **102**, 729–736 CrossRef PubMed

88 Sriramula, S., Cardinale, J. P., Lazartigues, E. and Francis, J. (2011) ACE2 overexpression in the paraventricular nucleus attenuates angiotensin II-induced hypertension. Cardiovasc. Res. **92**, 401–408 CrossRef PubMed

89 Xia, H., Suda, S., Bindom, S., Feng, Y., Gurley, S. B., Seth, D., Navar, L. G. and Lazartigues, E. (2011) ACE2-mediated reduction of oxidative stress in the central nervous system is associated with improvement of autonomic function. PLoS ONE **6**, e22682 CrossRef PubMed

90 Diz, D. I., Garcia-Espinosa, M. A., Gegick, S., Tommasi, E. N., Ferrario, C. M., Ann Tallant, E., Chappell, M. C. and Gallagher, P. E. (2008) Injections of angiotensin-converting enzyme 2 inhibitor MLN4760 into nucleus tractus solitarii reduce baroreceptor reflex sensitivity for heart rate control in rats. Exp. Physiol. **93**, 694–700 CrossRef PubMed

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