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Protective Angiotensin Type 2 Receptors in the Brain and Hypertension

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Abstract

Purpose—The goal of this review is to assess the evidence that activation of angiotensin type 2 receptors (AT2R) in the brain can lower blood pressure, and possibly constitute an endogenous anti-hypertensive mechanism.

Recent Findings—Recent studies that detail the location of AT2R in the brain, particularly within or near cardiovascular control centers, meshes well with findings from pharmacological and gene transfer studies which demonstrate that activation of central AT2R can influence cardiovascular regulation. Collectively, these studies indicate that selective activation of brain AT2R causes moderate decreases in blood pressure in normal animals, and more profound anti-hypertensive effects, along with restoration of baroreflex function, in rodent models of neurogenic hypertension.

Summary—These findings have opened the door to studies that can: (i) assess the role of specific AT2R neuron populations in depressing blood pressure; (ii) determine the relevance of such mechanisms; (iii) investigate interactions between AT2R and depressor angiotensin-(1-7)/Mas mechanisms in the brain.

Keywords

Angiotensin type 2 receptor; Neurogenic Hypertension; Solitary Tract Nucleus; Paraventricular nucleus; Compound 21

2. Introduction

Decades of research has established that the octapeptide angiotensin II (Ang II) acts via its type 1 receptors (AT1R) in brain cardiovascular control centers to increase blood pressure,

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Compliance with Ethical Standards

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an effect that involves stimulation of sympathetic outflow and vasopressin secretion [1]. Chronic over activation of this Ang II/AT1R mechanism contributes significantly to the resistant or “neurogenic” hypertension that is characterized by sustained sympathoexcitation, and also to impaired baroreflex function [2], and much is known about the mechanisms involved [3, 4, 5]. Aside from the physiologically essential and pathologically deleterious Ang II/AT1R system, it is clear that other components and peptides of the renin-angiotensin system (RAS), collectively known as the protective RAS, can exert significant functional effects that are largely beneficial [6]. At present, this protective RAS consists of two major arms, the angiotensin converting enzyme (ACE)/Ang II/angiotensin type 2 receptor (AT2R) axis, and the angiotensin converting enzyme-2 (ACE2)/angiotensin-(1-7) [Ang-(1-7)/Mas receptor axis [6]. However, in the coming years it is likely that other components such as alamandine/Mas-Related G Protein-coupled Receptor D (MrgD) will be recognized as essential elements of the protective RAS [7]. Since the functions, including cardiovascular effects, of the ACE2/Ang-(1-7)/Mas receptor axis have been the subject of extensive reviews elsewhere [8, 9], here we will focus on the AT2R. It is apparent that stimulation of AT2R results in improvement of renal blood flow, natriuresis, enhanced baroreflex function, vasodilation, antifibrotic, anti-inflammatory and neuroprotective actions, to name a few [6,10,11,12]. However, until recently the potential CNS-mediated effects of Ang II/AT2R and Ang-(1-7)/Mas in blood pressure regulation and hypertension have been understudied, and certainly not well understood. Studies have now been published which indicate anti-hypertensive roles for AT2R within the brain. We will review these studies with a view to assessing their potential importance as anti-hypertensive mechanisms, and will also discuss questions that remain open. We begin with basic information about the AT2R, and their distribution within the brain.

3. AT2R: Characteristics and brain distribution/cellular localization

AT2R

Like the AT1R, the AT2R is a G-protein coupled receptor (GPCR) that belongs to the seven-transmembrane domain family of receptors [13]. It shares only ~34% identity at the amino acid level with the AT1R, and Ang II displays ~15-fold selectivity for AT2R vs. AT1R [14]. However, the AT2R is termed an “atypical” or “non-classical” GPCR because even though it does signal via G-protein (Gi)-dependent mechanisms, it does not affect the classical cAMP, phospholipase C (PLC) or calcium releasing intracellular signaling mechanisms [15]. Furthermore, activation of AT2R also induces G-protein independent intracellular signaling in neurons [15]. Knowledge of the distribution of AT2R in the brain has undergone significant evolution since this angiotensin receptor subtype was identified in 1989. The initial view, gained from receptor autoradiography, was three-fold [16,17,18,19]. *First*, that AT2R are expressed at very high levels in neonatal tissues (brain and non-brain), but that their expression is much more limited and localized in adults. *Second*, that the expression pattern of AT2R in mammals is fairly unique and is, on the whole, distinct from that of AT1R. *Third*, at that time the highest densities of AT2R in adult brain were found in areas such as the inferior olive, locus ceruleus, ventral septum, lateral septum, mediodorsal thalamic nuclei, medial amygdala, red nucleus, and medial geniculate nucleus, none of which are directly involved in the neural pathways that control blood pressure. While these

original findings are certainly correct, subsequent *in situ* hybridization studies revealed that the distribution of AT2R in the brain is more complex, including localization within the solitary tract nucleus (NTS) [20], an area that is well known to modulate baroreflex function and sympathetic outflow [21], amongst other functions. The view of AT2R distribution in the brain took a big step forward with the development of a transgenic AT2R reporter mouse by our group [••22]. The distribution of AT2R in the brains of these mice, supported by fluorescence *in situ* hybridization, not only confirmed earlier studies that AT2R are present in the NTS, but also provided a more discrete view of the cellular localization of AT2R in the brain. At a regional level, AT2R are present either within or adjacent to brain areas that are directly responsible for modulating sympathetic outflow and baroreflex function. For example, high concentrations of AT2R-containing neurons were found in the intermediate NTS (intNTS) and the area postrema (AP), AT2R neuron fibers were present in the rostral ventrolateral medulla (RVLM), and while no AT2R neuron cell bodies were found within the paraventricular nucleus of the hypothalamus (PVN), they were observed on the periphery of this sympathetic control center [••22, ••23]. It was also clear that the PVN contains an abundance of AT2R-positive fibers and terminals, some of which are derived from the peri-PVN AT2R neurons and others from nuclei such as the median preoptic nucleus (MnPO), which is important in metabolism and osmoregulation. Aside from their presence within or adjacent to brain cardiovascular control centers, AT2R are also present within areas that can exert indirect influences on blood pressure and heart rate, such as the dorsomedial hypothalamic nucleus and the amygdala [••22]. The other advantage that these AT2R reporter mice have provided is that it has been made possible to determine the cellular location of AT2R in the brain. For example, under normal conditions they are observed only on neurons, and not on astrocytes nor on microglia [••22]; it will be interesting to ascertain whether this cellular distribution changes under hypertensive conditions. Furthermore, it has been possible to determine the phenotype of AT2R-containing neurons at cardiovascular control centers. At the intNTS, the AT2R neurons are largely GABAergic, as are the AT2R neurons in the peri-PVN area that terminate in the PVN [••22, ••23]. There are also populations of AT2R-containing glutamate neurons in the intNTS and MnPO [••22].

Thus, it is now established that AT2R are positioned in or adjacent to areas of the brain that control sympathetic cardiovascular function; however, *location* does not prove a role in cardiovascular function, as these CNS nuclei contain multiple neuronal populations that serve multiple physiological roles beyond blood pressure control [21,24]. The following section will discuss the evidence that activation of AT2R in the brain can influence cardiovascular control and exert anti-hypertensive effects.

4. Brain AT2R, Cardiovascular effects and Hypertension

Unsurprisingly, potential cardiovascular effects mediated through AT2R in the brain were disregarded for many years. Functional studies had clearly demonstrated that the increases in blood pressure elicited by brain application of Ang II are AT1R-mediated [25,26], and in most cases, central application of an AT1R-blocker or AT1R-antisense lowered blood pressure in rodent hypertensive models [27,28,29]. Furthermore, based on early receptor autoradiographic studies, the predominant angiotensin receptor subtype within brain cardiovascular control centers was the AT1R, with little or no presence of the AT2R at those

sites [16,19]. However, the view that central actions of Ang II on blood pressure and hypertension are totally restricted to AT1R has undergone revision in recent years. The initial demonstrations that AT2R-knockout mice display enhanced pressor responsiveness to Ang II (and in one case increased baseline blood pressure) [30,31], and increased susceptibility to develop DOCA-salt hypertension [32], indicated a sympathoinhibitory or blood pressure-lowering role of AT2R, but the fact that these were global and not brain-specific knockouts did not directly indicate a role for these sites in the brain in blood pressure control. Later studies began to directly suggest a role for *central* AT2R in blood pressure control. For example, Li et al. [33] demonstrated that the pressor effect of centrally-injected Ang II was enhanced when co-injected with an AT2R antagonist, and Matsuura et al. [34] found that AT2R in sympathetic neurons in the RVLM exert an antagonistic role against AT1R in controlling neuronal activity. Furthermore, short-term adenoviral-mediated over-expression of AT2R in the RVLM elicited a decrease in baseline blood pressure and norepinephrine excretion [35], linking AT2R within a specific cardiorespiratory brain nucleus to blood pressure control and sympathetic outflow. The same group followed this up with a study which demonstrated that AT2R in the RVLM exert an inhibitory effect on sympathetic outflow, and that down-regulation of these AT2R in chronic heart failure contributes to sympathoexcitation in this disease [36]. The availability of both non-peptide [Compound 21; C21]- and peptide [CGP 42112A; CGP] AT2R-selective agonists [14] has enabled a number of different groups of investigators to demonstrate that central application of either of these agents can exert blood pressure lowering and sympathoinhibitory effects. For example, infusion of C21 into the cerebroventricles (ICV) of *normotensive* rats elicits moderate decreases in blood pressure and suppresses sympathetic activity, via a nitric oxide (NO)-dependent mechanism [37]. Also in *normotensive rats*, ICV infusion of CGP enhances the renal sympathoinhibition in response to acute volume expansion, but this effect was not dependent on NO [38]. Interestingly, direct microinjection of CGP into the intermediolateral cell column of the spinal cord in normotensive rats produced a more significant fall in blood pressure, as well as sympathoinhibition [39]. The cardiovascular actions of ICV-infused C21 appear more profound under disease conditions: it suppresses sympathetic outflow by improving baroreflex sensitivity in rats with heart failure [40]; in SHR, it lowers blood pressure through sympathoinhibition, and improves spontaneous baroreflex sensitivity, via an NO-dependent mechanism [41]; it lowers blood pressure in female DOCA-salt hypertensive rats via a mechanism that includes generation of the anti-inflammatory cytokine interleukin-10 [42]. Furthermore, ICV infusions of the AT2R antagonist PD123,319 augmented the increase in blood pressure in female DOCA-salt hypertensive rats (no effect of PD123,319 was observed in male rats) [43]. These pharmacological studies using C21, CGP and PD123,319 are supported by data which demonstrate that chronic AAV2-mediated increased expression of AT2R in the NTS of 2 kidney-1 clip hypertensive rats decreases blood pressure and restores baroreflex sensitivity [44]. Similar over expression of AT2R in the NTS of SHR with established hypertension restored baroreflex sensitivity [45].

Thus, on the whole, the pharmacological and gene transfer investigations that have followed the initial AT2R knockout studies are important in that they revealed beneficial actions of these receptors to lower blood pressure and restore baroreflex function in hypertensive

models. These actions of AT2R make sense when considering their anatomical distribution in the brain, as described under section 3. Nonetheless, so far, studies on central AT2R and blood pressure control have only skimmed the surface in understanding the importance of these sites to a potential anti-hypertensive mechanism. Many knowledge gaps remain to be filled, with one glaring hole being the lack of a complete picture of the cell type location and expression of AT2R within or near cardiovascular control centers, under normal and hypertensive conditions. Such information would allow understanding of AT2R-effects on specific neuronal systems within cardiovascular control centers, as opposed to assessing effects at the gross regional or nucleus level. Towards this point, the AT2R reporter mouse described above enables identification of the cell phenotype on which AT2R exist in the brain [••22], and because of that has begun to allow uncovering of the neuronal circuitry/mechanisms that are responsible for their cardiovascular actions. For example, the major localization of AT2R in the intNTS is on GABA neurons [••22,46]. When taking into account that GABA acts within the intNTS to exert a powerful increase in blood pressure [47], an effect that is exacerbated in hypertension [48,49], the localization of AT2R on GABA neurons in the intNTS raises the idea that they can exert depressor effects by suppressing GABA activity. On a different note, the AT2R reporter mouse also revealed that AT2R-containing neurons terminate within the PVN, synapsing onto both pre-autonomic neuron cell bodies and onto AVP magnocellular neurons [••22, ••23]. This raised the possibility that AT2R can influence blood pressure through these connections, and in a recent study we demonstrated that C21-mediated activation of AT2R on GABA-neuron terminals that synapse onto AVP neurons in the PVN leads to an inhibition of those AVP neurons, and a reduction in plasma AVP [••23]. Therefore, knowledge of the discrete localization of AT2R in the brain was used to drive studies which revealed a specific cellular action of AT2R (inhibition of AVP neurons) that can lead to alterations in cardiovascular function. Future studies that combine AT2R genetically-modified mouse models with state-of-the-art optogenetic or DREADD techniques to selectively activate/inhibit AT2R containing neurons may enable in-depth analysis of the contribution of AT2R in cardiovascular control centers to blood pressure control and hypertension. While much progress has been made that supports a role for brain AT2R in blood pressure control and hypertension, many fundamental issues remain to be resolved. These include developing an understanding of the regulation of expression of AT2R (up- or down) at brain cardiovascular control centers during hypertension, determining the effects of gender on AT2R-induced anti-hypertensive mechanisms, and revealing the cellular mechanisms through which AT2R elicit decreases in blood pressure. However, from a broader perspective important questions such as the physiological and pathological relevance of the AT2R depressor/anti-hypertensive mechanism remain. This, and other questions, such as the relationship between AT2R depressor/anti-hypertensive mechanisms with similar Mas receptor-mediated mechanisms, and with Ang II/AT1R pro-hypertensive pathways are discussed in the following section.

5. Open questions

If brain AT2R are anti-hypertensive, why do they not prevent neurogenic hypertension?

The data that are available to this point might suggest that AT2R within or close to cardiovascular control centers such as the NTS, RVLM and PVN constitute a mechanism that combats the signals that drive sympathoexcitation, increased blood pressure, and hypertension. However, this clearly does not happen; the AT2R-dependent mechanisms either fail to operate, or are inadequate in stopping the increases in blood pressure during hypertension. There are a number of possible explanations for this failure. Hypertension is a multifactorial disease, and a single anti-hypertensive mechanism such as AT2R activation will likely not offset the actions of the many pro-hypertensive signals. Furthermore, such a mechanism would rely upon and compete for endogenous Ang II, which also stimulates AT1R at the above regions to exert powerful pro-hypertensive effects. However, the potential is there for AT2R to be an antihypertensive mechanism, certainly as their expression is elevated in the hypothalamus [50] and in the intNTS [51] during hypertension. One way of tipping the balance towards AT2R and engaging its anti-hypertensive effects would be with the use of a selective agonist (such as C21) for these sites. It is evident that this strategy has worked in a number of studies [•40–•42].

Do brain AT2R cooperate with Mas receptors to decrease blood pressure?

There is substantial evidence that Ang-(1-7), acting via Mas, can influence blood pressure via the brain, with both region-specific depressor and pressor effects being reported [8, •9]. Furthermore, immunohistochemical studies have revealed the presence of Mas in cardiovascular control centers of the brain, including the NTS, PVN, RVLM, caudal ventrolateral medulla and supraoptic nucleus [52]. A similarity between the actions of Ang-(1-7) and AT2R agonists on blood pressure concerns the effects obtained after ICV administration of these agents. Under normotensive conditions, ICV Ang-(1-7) fails to alter blood pressure, and depressor effects of AT2R agonists are weak. However, in several hypertensive models both Ang-(1-7) and AT2R agonists can exert powerful antihypertensive effects [8, •9, •40–•42]. Considering that they are both protective parts of the RAS, it is pertinent to ask whether they interact or cooperate in brain cardiovascular control centers to lower blood pressure? At present, this is difficult to answer, as it is now known that, as well as Mas, Ang-(1-7) can also bind to AT2R, albeit with low affinity [14,53]. Furthermore, there have been no studies which have assessed the cellular co-expression of Mas and AT2R in brain cardiovascular control centers. Nonetheless, a very recent article demonstrates dimerization between Mas and AT2R [54], and so their potential cooperation at the receptor level to lower blood pressure cannot be excluded.

Do brain AT2R directly antagonize the effects of brain AT1R on blood pressure?

It is established that over activity of brain Ang II/AT1R at regions such as the PVN, RVLM and intNTS makes a significant contribution to neurogenic hypertension [2], via mechanisms that include induction of chronic sympathoexcitation and neuroinflammation [3–5, 55]. The fact that AT2R exert anti-hypertensive actions raises the question of whether there are direct antagonistic effects between AT2R and AT1R on the same cells or via the same neural circuits. Based upon data from the AT2R reporter mouse, there is little overlap between the

cellular expression of AT2R and AT1R at the PVN, NTS, MnPO and AP, though the RVLM was not assessed [••22]. At the PVN, AT1R-containing neurons are localized to the neuroendocrine areas of this nucleus, and there were no AT1R-transcripts observed in the peri-PVN AT2R neurons [••22,56]. At the NTS, there was only ~9% co-expression of AT1R transcripts in AT2R-neurons [••22]. Furthermore, there was no detectable expression of AT2R on microglia or astrocytes *in vivo*, at least under normotensive conditions [••22]. Based on this, it is unlikely that (at least within the NTS) the opposing actions of AT2R and AT1R involve direct antagonistic effects at the same cells, whether neurons or glia. However, it will be essential to assess the cellular location of these sites under hypertensive conditions, to determine (for example) if there is increased expression of AT2R on AT1R-containing neurons or on inflammatory glial cells, during persistent high blood pressure. It is more likely that AT2R influence the same neuronal circuitry as AT1R, albeit via different mechanisms. For example, it is well-known that AT1R activation (likely pre-synaptic) leads to activation of post-synaptic pre-autonomic neurons in the PVN [57,58]. The presence of AT2R neuron terminals adjacent to the pre-autonomic neurons in the PVN [••22] puts them in a position to influence AT1R-mediated sympathoexcitation, though functional interactions will only be proven through further experimentation.

6. Conclusions

There is a growing body of evidence that when activated, AT2R within or near cardiovascular control centers can lower blood pressure. The data so far are consistent with the following scenario: that under *normotensive* conditions, AT2R within brain cardiovascular control centers such as the intNTS provide a mechanism for tempering increases in blood pressure and reduced baroreflex function; under *hypertensive* conditions, even with their increased expression, the depressor actions caused by endogenous Ang II at AT2R are not enough to offset the pro-hypertensive influences, and it requires a selective and potent AT2R agonist to fully activate the protective AT2R mechanisms. These ideas are represented as a diagram in Figure 1, which also includes the potential relationship to AT1R and Mas mechanisms. The current availability of AT2R [••22] transgenic reporter mice has provided a novel view of the location of these receptors at the discrete cellular level with cardiovascular control centers of the brain. This information will allow for targeting specific AT2R-containing neural circuits in the brain and determining their influence on blood pressure and hypertension.

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- Of major importance

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Figure 1. Hypothetical model

(i) Under *normotensive* conditions, AT2R within brain cardiovascular control centers provide a mechanism for tempering increases in blood pressure and reduced baroreflex function and, thus, are in equivocal ‘balance’ with brain pressor mechanisms. (ii) Under *hypertensive* conditions, despite the increased expression of AT2R, the depressor actions caused by endogenous Ang II at this receptor are not sufficient to offset the pressor influences; the consequence is elevated blood pressure. (iii) Addition of a selective and potent AT2R agonist (e.g., Compound 21 [C21]) to fully activate the protective AT2R mechanisms tips the balance back, such that ‘normal’ blood pressure is restored.