HHS Public Access

Author manuscript

Curr Hypertens Rep. Author manuscript; available in PMC 2021 July 13.

Published in final edited form as:

Curr Hypertens Rep. ; 22(7): 48. doi:10.1007/s11906-020-01062-0.

Brain Angiotensin Type-1 and Type-2 Receptors in Physiological and Hypertensive Conditions: Focus on Neuroinflammation

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Abstract

Purpose of Review.—To review recent data that suggest opposing effects of brain angiotensin type-1 (AT_1R) and type-2 (AT_2R) receptors on blood pressure (BP). Here, we discuss recent studies that suggest pro-hypertensive and pro-inflammatory actions of AT_1R, and anti-hypertensive and anti-inflammatory actions of AT_2R. Further, we propose mechanisms for the interplay between brain angiotensin receptors and neuroinflammation in hypertension.

Recent Findings.—The renin-angiotensin system (RAS) plays an important role in regulating cardiovascular physiology. This includes brain AT_1R and AT_2R, both of which are expressed in or adjacent to brain regions that control BP. Activation of AT_1R within those brain regions mediate increases in BP and cause neuroinflammation, which augments the BP increase in hypertension. The fact that AT_1R and AT_2R have opposing actions on BP, suggests that AT_1R and AT_2R may have similar opposing actions on neuroinflammation. However, the mechanisms by which brain AT_1R and AT_2R mediate neuroinflammatory responses remain unclear.

Summary.—The interplay between brain angiotensin receptor subtypes and neuroinflammation exacerbates or protects against hypertension.

Keywords

Brain angiotensin receptors; AT_1R; AT_2R; neuroinflammation; microglia; hypertension

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Conflict of Interest

The authors declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

The renin-angiotensin system (RAS) plays an important regulatory role in cardiovascular physiology [1]. This involves the actions of angiotensin II (Ang II) on angiotensin II type 1 (AT_1R) and type 2 (AT_2R) receptors within or near the brain cardiovascular control centers [2, 3]. AT_1R-mediated effects of Ang II within those centers result in sympatho-excitaion and vasopressin and corticosterone secretion, leading to increases in blood pressure (BP) [4–6, 7•]. Importantly, those mechanisms contribute to neurogenic hypertension, which is characterized by chronic sympatho-excitation [8, 9]. On the other hand, brain AT_2R are thought to elicit opposing effects to AT_1R [10, 11•, 12], and are suggested to be protective against hypertension [3]. AT_2R-mediated effects of Ang II result in sympatho-inhibition and attenuate vasopressin secretion, leading to decreases in BP [13, 14••, 15, 11•, 16]. These actions are consistent with the anatomical localization of both AT_1R and AT_2R in the brain [17, 18, 19••, 20••]. AT_1R are found within the cardiovascular control centers of the brain, including the paraventricular nucleus of the hypothalamus (PVN), nucleus of the solitary tract (NTS), area postrema (AP), and rostral ventrolateral medulla (RVLM) [17, 18, 20••]. While AT_2R are expressed within or adjacent to the cardiovascular centers containing AT_1R, it is evident that these angiotensin receptor subtypes are largely expressed by distinct cell populations [17, 18, 19••, 20••]. This suggests distinct brain circuitry and mechanisms by which AT_1R and AT_2R oppose one another in their regulation of cardiovascular physiology in hypertension.

Chronic local inflammation in cardiovascular control centers of the brain contributes to neurogenic hypertension [21, 22, 23•]. This includes the activation of microglia [22, 24], upregulation of pro-inflammatory cytokines and chemokines [25–27, 23•], and recruitment of circulating immune cells [28, 27, 29]. This local pro-inflammatory state results in sustained sympatho-excitaion, mediating prolonged increases in BP [23•, 25]. Furthermore, the pro-hypertensive actions of AT_1R involve the stimulation of local pro-inflammatory mechanisms [29, 23•, 30, 31], whereas the protective effects of AT_2R appear to include anti-inflammatory mechanisms [32, 14••, 33]. However, we recently found that both AT_1R and AT_2R at or near brain cardiovascular control centers are exclusively expressed by neurons and not by glia in both naïve and hypertensive rats and mice [20••]. This might suggest that both pro-hypertensive AT_1R and anti-hypertensive AT_2R induce respective pro-inflammatory and anti-inflammatory effects via local paracrine activity, possibly through the neuronal secretion of chemokines and cytokines that influence inflammatory cells and glia [34, 20••].

In the present review, we discuss evidence supporting the pro-hypertensive/pro-inflammatory effects of Ang II on neurons containing AT_1R, and its anti-hypertensive/anti-inflammatory effects on neurons containing AT_2R, in both normal and hypertensive conditions. Further, we propose mechanisms by which AT_1R and AT_2R induce pro-inflammatory and anti-inflammatory effects respectively.

The Renin-Angiotensin System (RAS)

The initial steps in the discovery of RAS began with the experiments performed by Robert Tigerstedt and Per Bergman at the Karolinska Institute, who demonstrated that the injection of rabbit kidney extract into naïve rabbits increased BP [35]. Those experiments laid out the foundations for the classical arm of the RAS. Under normal physiological conditions the secretion of renin by the kidneys acts to convert angiotensinogen into angiotensin I, which is then converted to Ang II by angiotensin converting enzyme (ACE) [1, 36, 37]. Circulating Ang II acting via AT_1R mediates vasocontraction, adrenal steroids secretion, vasopressin release, sodium reabsorption, and decreased renal blood flow, to ultimately increase blood volume and arterial BP [38, 39, 36]. For example, pressor responses to systemically infused Ang II are absent in mice lacking AT_1R [40]. Thus, the classical Ang II – AT_1R axis acting to increase BP is pro-hypertensive. In fact, one of the many rodent models of hypertension involves the continuous systemic infusion of Ang II, where hypertension is established one week following Ang II infusion [41]. Whereas, the systemic infusion of the AT_1R antagonist – losartan reduces BP in hypertension, which is consistant across many species, including hypertensive humans [42–44].

More recently, an alternative component of RAS was identified as the protective arm of the RAS [45, 3]. This protective arm includes the action of Ang II on AT_2R which are thought to counteract many of the actions of AT_1R [10]. This was first demonstrated in AT_2R-knockout mice which displayed an enhanced pressor responsiveness to Ang II, indicating a BP lowering effect of AT_2R [46, 47]. Indeed, experiments in isolated vessels from various vascular beds demonstrate a vasodilatory effect of AT_2R [48–50]. Furthermore, the *in vivo* selective pharmacological stimulation of AT_2R by agonists, such as Compound 21 (C21) and CGP42112, mediated a BP lowering effect under the blockade of AT_1R [51, 52]. More intriguingly, the infusion of the AT_2R-agonist C21 reduced BP in a rodent model of Ang II-induced hypertension [53, 54]. These studies indicate that the systemic activation of AT_2R can lower BP only when AT_1R are blocked, or when animals are hypertensive. Nonetheless, this suggests that the alternative/protective arm of RAS plays an anti-hypertensive role through Ang II – AT_2R axis.

While the evidence discussed suggests that these angiotensin receptor subtypes induce opposing effects in regulating cardiovascular physiology under normal physiological conditions, the evidence also suggest an important counter-regulatory role for these receptors in mediating hypertension. As autonomic dysfunction leading to chronic sympatho-excitation is a hallmark of hypertension [8, 9], this raises the question of whether brain AT_1R and AT_2R can act on brain circuitry to exert counter-regulatory effects on the sympathetic outflow to the cardiovascular organs.

The Brain Renin-Angiotensin System

Complex neural networks spanning a number of specialized brain structures are directly involved in controlling and regulating sympathetic outflow to the cardiovascular organs [55]. This includes a number of circumventricular organs in the forebrain and the brainstem that lack a functional blood brain barrier and can directly sense factors in systemic circulation [56]. The subfronical organ (SFO) in the forebrain, can sense circulating Ang II in systemic

circulation, and it projects to the pre-autonomic neurons of the paraventricular nucleus of the hypothalamus (PVN). These PVN pre-autonomic neurons project to the rostral ventrolateral medulla (RVLM) and to the sympathetic preganglionic neurons of the interomediolateral column of the spinal cord (IML) [4, 57, 55]. Similarly, the area postrema (AP) in the brainstem is another circumventricular organ that projects to the pre-autonomic neurons of the RVLM [58, 59]. Finally, the nucleus of the solitary tract (NTS) in the brainstem can directly sense cardiovascular function through afferents arising from the aortic arch and the carotid sinus which input on the NTS [60]. Those autonomic brain regions that sense, control, and regulate cardiovascular physiology are known as cardiovascular control centers, where Ang II can regulate cardiovascular physiology via acting on those regions [4, 61]. For example, circulating Ang II can impact the brain via actions at AT_1R-containing neurons in the SFO that innervate preautonomic (RVLM – projecting) neurons of the PVN to increase sympathetic nerve activity and BP [4, 5]. Similarly, circulating Ang II can increase BP by acting on AT_1R localized to RVLM – projecting neurons of the AP [62, 63]. Furthermore, in hypertension the blood brain barrier becomes increasingly permeable in highly vascularized brain regions that usually comprise of an intact blood brain barrier (under normal physiological conditions), allowing circulating Ang II to act on AT_1R in regions that include the PVN [30]. In addition, brain-derived Ang II may exert actions at AT_1R in the PVN, NTS and RVLM [45]. Thus, Ang II can regulate cardiovascular physiology by acting on brain circuitry that controls and regulates the sympathetic outflow to cardiovascular organs.

Brain AT_1R in Physiological and Hypertensive Conditions

The distribution and the roles of brain AT_1R are well-characterized and defined in the literature. The discovery of angiotensin receptor subtypes that revealed the location of AT_1R within the brain was primarily determined via receptor autoradiography [17, 64, 65]. These studies demonstrated a wide distribution of AT_1R expression in the brain, including high densities in the cardiovascular control centers. These views on the distribution of AT_1R in the brain were recently confirmed with the development of AT_1R-eGFP transgenic reporter mouse [66], and refined with the recent development of our AT_1R-tdTom transgenic reporter mouse [20••]. High densities of AT_1R are found in the SFO, PVN, AP, NTS and RVLM [66, 20••]. Interestingly, AT_1R are almost exclusively localized to neurons rather than to microglia or astrocytes in those cardiovascular regions of the brain [20••, 17]. Furthermore, AT_1R appears to be primarily expressed on glutamatergic neurons [7•, 20••]. This suggests that AT_1R can directly influence the neurons of the cardiovascular centers to elicit sympatho-excitation and increase BP. For example, the actions of Ang II on AT_1R in the SFO [4, 5], PVN [6, 7•], AP [67, 68], and IML [69] result in sympathetically mediated increases in BP, and the modulation of the baroreflex and BP via acting on the NTS [70, 71]. This therefore suggests a pro-hypertensive role that the brain Ang II – AT_1R plays during hypertension. In fact, the mRNA expression levels of AT_1R in the RVLM and the NTS were higher in hypertensive rats in comparison to normotensive rats [72, 73]. Furthermore, the administration of the AT_1R antagonist losartan into the brain reduces BP in hypertensive rats [72, 74]. Evidence discussed here supports a role for brain AT_1R in maintaining cardiovascular physiology under normal physiological conditions. In hypertension, the increased expression of AT_1R in the brain augments the effects of their stimulation, contributing to the severe increases in BP that are observed.

Brain AT_2R in Physiological and Hypertensive Conditions

While the distribution and the role of brain AT_1R is well characterized, only recently did investigators begin to uncover the roles of brain AT_2R. When the discovery of angiotensin receptor subtypes was made in the late 1980's, the location of AT_2R and AT_1R within the brain was primarily determined via receptor autoradiography or membrane binding studies [75, 76]. Based on these studies it was demonstrated that the expression of AT_2R was high and very widespread in neonatal tissues, including in the brain [75, 76]. In contrast, the studies indicated that in adult brain AT_2R expression is more limited and localized, and largely non-overlapping with that of AT_1R. For example, high densities of AT_1R were shown to be present in cardiovascular centers of the brain, which included the SFO, PVN, AP, NTS, and RVLM [17, 64, 65]. The highest densities of AT_2R, on the other hand, were shown to be present in areas such as the inferior olive, the septum and the medial geniculate nucleus, none of which have direct involvement in blood pressure control [75, 76]. These views on the distribution of AT_2R in the brain were largely confirmed, but also somewhat revised by early traditional autoradiographic *in situ* hybridization (ISH) studies, which revealed the presence of AT_2R mRNA within the NTS of adult rats [17].

By using the recent advent of newer technologies, namely the development of an AT_2R-eGFP transgenic reporter mouse and fluorescence ISH for the detection of AT_2R mRNA, we were able to better define the distribution of AT_2R within the brain, as the use of those technologies have enabled more sensitive and discrete cellular localization of AT_2R within the brain [19••]. Our AT_2R-eGFP reporter mouse provided a number of major advances. 1) It revealed a much more detailed regional pattern of AT_2R-positive cells in the adult brain, results supported by fluorescence ISH. 2) It revealed that in normotensive mice AT_2R are exclusively localized to neurons within the cardiovascular control centers that were assessed, and are not present on microglia or astrocytes. While the latter observation supports the findings of an earlier ISH study [17], a number of studies indicate the presence of AT_2R on astrocytes and microglia in culture [31, 77, 78]. 3) It enabled phenotyping of the neurons that contain AT_2R [19••]. Results from the AT_2R-eGFP reporter mice that are of particular interest are those which demonstrate the localization of AT_2R-positive cells within or near to brain areas that have direct influence on sympathetic outflow and BP control. For example, use of this mouse not only confirmed the presence of AT_2R containing neurons in the intermediate NTS, but also demonstrated that these neurons are primarily GABAergic [19••]. The reporter mouse also revealed a heavy concentration of AT_2R-positive neurons in the AP, as well as AT_2R neuronal fibers in the RVLM and PVN [19••]. Interestingly, a number of the AT_2R-positive neuronal fibers and terminals in the PVN are derived from AT_2R containing GABAergic neurons that surround this nucleus [11•]. This suggests that AT_2R are primarily expressed by the GABAergic neurons in the cardiovascular centers, which possibly allows them to elicit sympatho-inhibition and decreases in BP.

The first evidence that stimulation of brain AT2R lowers BP, came from pharmacological studies which demonstrated that the AT1R-mediated pressor responses to intracerebroventricularly applied Ang II were amplified in the presence of the AT2R antagonist PD123319 [79]. More evidence of a sympatho-inhibitory and BP lowering effects of AT2R has since emerged using the selective AT2R agonists, CGP42112 or C21. For

example, the chronic brain intracerebroventricular infusion of C21 in conscious rats resulted in sympatho-inhibition, leading to a reduction in BP [15]. Similarly, the local administration of CGP42112 or C21 into the RVLM and the IML led to sympatho-inhibition and BP lowering effects [16, 80, 69]. Collectively these findings suggested an anti-hypertensive effect of brain AT2R. Indeed, the chronic intracerebroventricular infusion of C21 in spontaneously hypertensive rats and in DOCA-salt induced hypertension reduced sympathetic nerve activity and BP [13, 14]. Anti-hypertensive effects are also observed with the increased expression of AT2R in the RVLM and the NTS of hypertensive rodents, which leads to decreased BP [80–82]. The evidence discussed supports a protective role for brain AT_2R in regulating cardiovascular physiology under physiological and hypertensive conditions.

Relative Distribution & Cellular localization of Brain Angiotensin Receptor Subtypes

In a more recent study, we have further refined the location and distribution of both AT_1R and AT_2R within the brain [20••]. Firstly, using a novel AT_1R/AT_2R dual reporter mouse we demonstrated that within the PVN and the NTS, AT_1R and AT_2R are exclusively expressed by neurons rather than microglia or astrocytes. Further, this study provides evidence that there is no re-distribution of AT_1R and AT_2R from neurons to astrocytes or microglia in cardiovascular control centers during sustained hypertension induced by DOCA-salt administration [20••]. Secondly, using the AT_1R/AT_2R dual reporter mouse, it was possible to perform a detailed analysis of the relative locations of AT_1R and AT_2R within brain areas of male and female mice that are either directly or indirectly involved in cardiovascular regulation. These studies revealed that there are greater numbers of AT_2R-positive cells than AT_1R-positive cells in both the NTS and AP, with no differences between female and male mice (Table 1) [20••]. Furthermore, it is apparent that AT_1R and AT_2R are localized primarily to different populations of neurons, with the highest levels of colocalization observed in the AP and NTS [20••]. For example; AT_1R are mainly expressed on glutamatergic neurons in the PVN, whereas AT_2R are expressed on GABAergic neurons that surround the PVN with their fibers inputting on the PVN (Table 1). Thus, based on the mostly distinct localization of AT_1R and AT_2R to separate neurons in brain regions that influence the cardiovascular system, it is reasonable to speculate that contrasting functional effects of these receptors are mediated through different neuronal circuits; however, the possibility that there are intracellular interactions stimulated by these receptors on the same neurons that influence cardiovascular regulation cannot be entirely excluded at this time.

The Interplay Between Brain Angiotensin Receptors and Neuroinflammation

Neuroinflammation is a hallmark of neurogenic hypertension [22, 27]. This involves the upregulation of pro-inflammatory cytokines and the downregulation of anti-inflammatory cytokines in the cardiovascular control centers of the brain. In Ang II – induced hypertension, the mRNA levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, are increased by at least 3-fold in the PVN, whereas the levels of anti-inflammatory cytokines, such as IL-10, are reduced [22]. These inflammatory processes in the cardiovascular centers of the brain mediate sympatho-excitation and increases in BP [23•,

25]. The administration of TNF-α or IL-1β into the SFO increases renal sympathetic nerve activity and BP [83•]. Similarly, in the PVN and the AP, TNF-α and IL-1β mediate increases in renal and cardiac sympathetic nerve activity; leading to elevations in BP [23•, 25]. More intriguingly, the blockade of receptors for TNF-α or IL-1β in the SFO [84], PVN [85, 86], or AP [25] reduces BP in hypertensive rats. Thus, neuroinflammation or the upregulation of pro-inflammatory cytokines in the cardiovascular centers of the brain results in sympathetically – mediated increases in BP, establishing a hypertensive phenotype.

The major source of pro-inflammatory cytokine production in the brain is the resident immune cells of the central nervous system (microglia and astrocytes) [22, 87]. In hypertension, microglia in the cardiovascular centers become activated and exhibit a pro-inflammatory phenotype; leading to the local upregulation and secretion of pro-inflammatory cytokines [22]. By preventing the activation of microglia, the levels of pro-inflammatory cytokines are significantly reduced in regions such as the PVN, leading to the attenuation of high BP in hypertensive rats [22]. This local pro-inflammatory state, established by microglia, is capable of directly activating the pre-autonomic neurons [25]. Those pre-autonomic neurons not only play a role in mediating sympatho-excitation, but can contribute to the local inflammatory state by secreting pro-inflammatory chemokines [27]. For example, the application of Ang II to primary hypothalamic neurons, increases the mRNA and protein levels of the chemokine CCL2 in cell culture media [27]. CCL2 is a pro-inflammatory chemokine, upregulated in hypertensive rodents [27], and is capable of making the blood brain barrier more permeable [88, 89], to recruit and facilitate the infiltration of peripherally circulating immune cells [27, 28]. Infiltrating peripheral immune cells are another source of pro-inflammatory cytokine production [90]. Thus, cytokine production and secretion by infiltrating immune cells further activate pre-autonomic neurons and microglia; exacerbating the pro-inflammatory state and augmenting the sympathetic outflow and BP in hypertension [26, 91]. This vicious positive feedforward cycle between neurons, microglia, and infiltrating immune cells, results in a sustained pro-inflammatory state in the cardiovascular centers of the brain that leads to the severely increased BP in hypertension.

The RAS is a major contributor to the sustained pro-inflammatory state in the brain. For example, a single systemic administration of Ang II is capable of inducing a prolonged activation of PVN microglia, peripheral immune cell activation and mobilization, and activating the neurons of the cardiovascular centers of the brain, leading to an increased BP [29]. More intriguingly, the blockade of AT_1R in the SFO [83•] or in the PVN [23•] prior to the administration of TNF-α or IL-1β, completely abolishes the sympathetic nerve activity and BP responses induced by both pro-inflammatory cytokines.

Brain Angiotensin Receptors and Neuroinflammation

AT_1R as Pro-inflammatory—The pro-inflammatory effects of AT_1R are well-documented in the literature. For example, AT_1R are involved in the production of pro-inflammatory cytokines both *in vitro* and *in vivo* [92]. In hypertensive conditions, the blockade of AT_1R significantly reduces the levels of pro-inflammatory cytokines in peripheral tissues, such as the aorta, and in the brain cardiovascular centers, such as the PVN

[93, 94]. This blockade of AT_1R that results in a reduced expression of pro-inflammatory cytokines leads to the attenuation of the increased renal sympathetic nerve activity and BP [95]. In addition, the selective deletion of AT_1R in the PVN reduces the expression of pro-inflammatory cytokines and lowers BP [96•, 97•]. The expression of pro-inflammatory cytokines is regulated by the nuclear transcription factor NF-κB [98]. Interestingly, the blockade of AT_1R in hypertensive rats reduces the expression of NF-κB [98, 99]. This therefore suggests that AT_1R can regulate the expression of NF-κB, and thus can regulate the production of pro-inflammatory cytokines. Furthermore, this link between AT_1R, NF-κB, and pro-inflammatory cytokines is demonstrated within the PVN [95], supporting the notion that AT_1R-containing neurons within the cardiovascular control centers can directly signal to the production of pro-inflammatory cytokines. In fact, Ang II acting on AT_1R in the PVN results in the upregulation of pro-inflammatory cytokines, leading to sympatho-excitaion and BP elevation [100, 29]. These studies demonstrate a pro-inflammatory role that AT_1R play, which can exacerbate the local inflammatory state in the cardiovascular centers of the brain and augment BP in hypertension.

AT_2R as Anti-inflammatory—While AT_2R are thought to be protective and play a counter-regulatory role to AT_1R, only a handful of studies demonstrate an anti-inflammatory role of AT_2R. Those studies utilize pharmacological AT_2R agonists and antagonists to reveal the anti-inflammatory effects of AT_2R (Table 2). In rodent models of neuroinflammation and autoimmune disease, the activation of AT_2R by the intraperitoneal administration of CGP42112 or C21 reduced microglia activation, the number of infiltrating immune cells, and the expression of pro-inflammatory cytokines, while increasing the levels of anti-inflammatory cytokines such as IL-10, within the central nervous system [101, 102]. In cardiovascular disease, the administration of C21 following middle cerebral artery occlusion (stroke), reduced microglia activation and peripheral immune cell infiltration [33, 32]. The protective anti-inflammatory effect of AT_2R stimulation improved cognitive function and survival following stroke [33, 32]. Furthermore, DOCA-salt induced hypertension in female rats was augmented by intracerebroventricular administration of AT_2R antagonist – PD123319 [14••]. The blockade of AT_2R in those hypertensive rats further exacerbated the levels of pro-inflammatory cytokines in the PVN, and further reduced the levels of anti-inflammatory cytokines [14••]. These studies suggest that; (i) AT_2R induce anti-inflammatory effects, (ii) those anti-inflammatory effects protect against cardiovascular disease, and (iii) anti-inflammatory effects of AT_2R may be linked to the upregulation of anti-inflammatory cytokines. Thus, as AT_2R oppose the actions of AT_1R, and AT_1R is demonstrated to have pro-inflammatory effects through regulating the production of pro-inflammatory cytokines, it is possible that AT2R exert anti-inflammatory effects via regulating the production of anti-inflammatory cytokines such as IL-10.

Mechanisms for the Interplay Between Brain Angiotensin Receptors and Neuroinflammation

While several studies seem to indicate that brain angiotensin receptors are expressed on glia [31, 77, 78], the use of the recent advanced technologies in our studies have enabled more sensitive and discrete cellular localization of AT_1R/AT_2R within the brain. These studies revealed that AT_1R and AT_2R are exclusively expressed by neurons rather than microglia or

astrocytes in the cardiovascular centers of the brain [19••, 20••]. In those studies, demonstrating AT_1R expression on microglia and astrocytes, Percoll density gradients were used to isolate microglia and astrocytes from the macro-dissected hypothalamus, followed by RT-PCR to demonstrate the presence of AT_1R in the isolated cells [31, 78]. However, it is extremely difficult to isolate only glia or dissect the hypothalamus with such procedures, and the isolates used in the above studies may have contained neurons and glia from brain tissue surrounding the hypothalamus. Nonetheless, the possibility that brain angiotensin receptors are expressed by microglia to regulate the inflammatory processes, cannot be excluded at this point, although further studies are warranted to validate and confirm the expression of brain angiotensin receptors by glia in vivo.

Our recent studies demonstrate that AT_1R are exclusively expressed by neurons within or adjacent to the cardiovascular control centers [19••, 20••]. Therefore, it is reasonable to speculate that AT_1R regulate inflammatory processes through local paracrine activity. We propose that the actions of Ang II on AT_1R mediate; (i) sympatho-excitation and increases in BP [4–6, 7•], (ii) upregulation of pro-inflammatory cytokines via NF-κB signaling [98, 99, 95], and (iii) secretion of pro-inflammatory chemokines such as CCL2 [27]. The upregulation of pro-inflammatory cytokines, such as TNF-α and IL-1β, is capable of activating neurons to further increase sympathetic nerve activity, BP, and pro-inflammatory cytokine production [23, 83•, 25]. Pro-inflammatory cytokines can activate microglia to exhibit a pro-inflammatory phenotype; leading to the further release of cytokines [22]. Furthermore, the upregulation of chemokines, such as CCL2, facilitates the activation and the recruitment of peripherally circulating immune cells to infiltrate cardiovascular centers of the brain [28, 26, 27]. Infiltrating peripheral immune cells are a major source of pro-inflammatory cytokine production, and will result in the further upregulation of pro-inflammatory cytokines [90]. This vicious positive feedback cycle – whereby Ang II acting on neuronal AT_1R mediates pro-inflammatory cytokine production; leading to the activation of microglia and immune cell recruitment to further upregulate pro-inflammatory cytokines, results in the establishment of a local chronic pro-inflammatory state (Fig. 1a). The sympatho-excitatory and pro-inflammatory effects of AT_1R augment sympathetic outflow and elevate BP as observed in hypertension. Therefore, we propose deleterious pro-hypertensive/pro-inflammatory effects of AT_1R in the brain cardiovascular control centers.

Newly emerging views suggest that brain AT_2R counter-regulate the actions of AT_1R on BP [10–12]. This notion is supported by our recent studies demonstrating that the neuronal populations expressing AT_2R are distinct from the populations that express AT_1R [19••, 20••]. Thus, it is reasonable to speculate that AT_1R and AT_2R regulate inflammatory processes through distinct neuronal circuits. We propose that the stimulation of AT_2R mediates; (i) sympatho-inhibition and BP lowering effects [16, 80, 13, 14••], and (ii) protective anti-inflammatory effects by increasing anti-inflammatory cytokine production [33, 32, 14••]. This proposition is supported by the fact that in the vast majority of studies that demonstrated BP lowering effects of AT_2R, chronic infusions of AT_2R agonists were required to observe such responses and the onset of the response was always delayed [15, 16, 80, 13, 14]. The delayed onset of the BP lowering effects of AT_2R may be related to duration required for the induction of the anti-inflammatory cascade. The activation of AT_2R is linked to the upregulation of the anti-inflammatory cytokine, IL-10, which induces anti-

inflammatory effects [14••, 102]. Those anti-inflammatory effects of IL-10 include inhibiting the activation of microglia, the recruitment of immune cells, and inhibiting the production of pro-inflammatory cytokines, such as TNF-α [103, 104]. Anti-inflammatory effects of IL-10 were observed in the PVN following the intracerebroventricular gene transfer of IL-10 in rats with acute myocardial infarction [105]. Furthermore, the intracerebroventricular [106] and PVN [22] administration of IL-10 reduced BP in hypertensive rats. More intriguingly, IL-10 inhibited the effects elicited by Ang II acting on AT_1R in hypothalamic neurons [107]. This indeed suggests that the anti-inflammatory actions of IL-10 (the secretion of which may well be spurred by AT_2R) counteract the actions of AT_1R. Therefore, it is reasonable to hypothesize that the stimulation of AT_2R results in the upregulation of IL-10, which in turn inhibits the activation of microglia, immune cell recruitment and the production of pro-inflammatory cytokines, establishing a local anti-inflammatory state that decreases BP (Fig. 1b). The sympatho-inhibitory and the anti-inflammatory effects of AT_2R reduce BP. Thus, we propose protective anti-hypertensive/anti-inflammatory effects of AT_2R.

More recent studies indicate that Ang II may directly act on microglia through different receptors, other than angiotensin receptors, to regulate inflammatory processes [31, 108, 109]. This involves a receptor that plays a major role in the innate immune response, and is termed toll-like receptor 4 (TLR4) [110]. *In vitro* studies demonstrate a link between Ang II and TLR4 in regulating pro-inflammatory cytokine production [109]. Furthermore, the central blockade of TLR4 seems to improve cardiac tissue inflammation in Ang II – induced hypertension [108]. Interestingly, TLR4 is expressed by microglia in the PVN, and the pro-inflammatory responses mediated by Ang II in the PVN are completely abolished in mice lacking TLR4 [31]. However, without further studies to validate the crosstalk between Ang II and TLR4, it is difficult to infer that Ang II can regulate the inflammatory processes by interacting with glial TLR4. It is possible that the pro-inflammatory cytokines released by neurons in response to Ang II – AT_1R act on TLR4 to activate microglia to induce a pro-inflammatory state. Thus, binding studies are warranted and are essential to validate and confirm this crosstalk between Ang II and TLR4.

In summary, we propose distinct neuronal circuitry that regulates the inflammatory processes in the cardiovascular centers of the brain. AT_1R exert pro-inflammatory actions through local paracrine activity that results in the upregulation of pro-inflammatory cytokines such as TNF-α and IL-1β, and subsequent increased blood pressure. In contrast, AT_2R mediate anti-inflammatory effects through the upregulation of anti-inflammatory cytokines such as IL-10, and these effects contribute to the AT_2R mediated fall in blood pressure (Fig. 1).

Closing Remarks

Emerging views suggest that brain AT_1R and AT_2R mediate counter-regulatory effects on blood pressure by acting on distinct neuronal circuits and via mechanisms that include respective pro- and anti-inflammatory actions.

Previous studies demonstrated:

- Sympatho-excitatory and pressor effects of AT_1R.
- Pro-hypertensive effects of AT_1R.
- Pro-inflammatory effects of AT_1R.
- Sympatho-inhibitory and depressor effects of AT_2R.
- Anti-hypertensive effects of AT_2R.
- Anti-inflammatory effects of AT_2R.

Our studies demonstrated:

- AT_1R and AT_2R are expressed within or adjacent to the brain cardiovascular centers.
- AT_1R and AT_2R are exclusively expressed on neurons rather than glia.
- AT_1R and AT_2R are predominantly expressed by distinct neuronal populations.

Therefore, studies discussed in this review suggest that AT_1R and AT_2R mediate their actions through distinct neuronal circuitry; (i) a deleterious sympatho-excitatory/pro-hypertensive/pro-inflammatory AT_1R circuit, and (ii) a protective sympatho-inhibitory/anti-hypertensive/anti-inflammatory AT_2R circuit.

Conclusion

The renin – angiotensin system plays an important regulatory role in cardiovascular physiology [1]. This involves actions of angiotensin II on its receptors within the brain [2, 3]. In the cardiovascular control centers of the brain, AT_1R and AT_2R are expressed on distinct neuronal populations [19••, 20••]. AT_1R mediate sympatho-excitation and blood pressure elevations [4–6, 7•], and the upregulation of pro-inflammatory cytokines/chemokines [98, 99, 95, 27], which further augment sympathetic outflow and blood pressure elevation [23•, 83•, 25]. In contrast, AT_2R mediate sympatho-inhibitory and blood pressure lowering effects [16, 11•, 80, 13, 14••], and the upregulation of anti-inflammatory cytokines [14••, 102]. Therefore, AT_1R mediate deleterious sympatho-excitatory/pro-hypertensive/pro-inflammatory effects that exacerbate hypertension, whereas AT_2R mediate protective sympatho-inhibitory/anti-hypertensive/anti-inflammatory effects that protect against hypertension.

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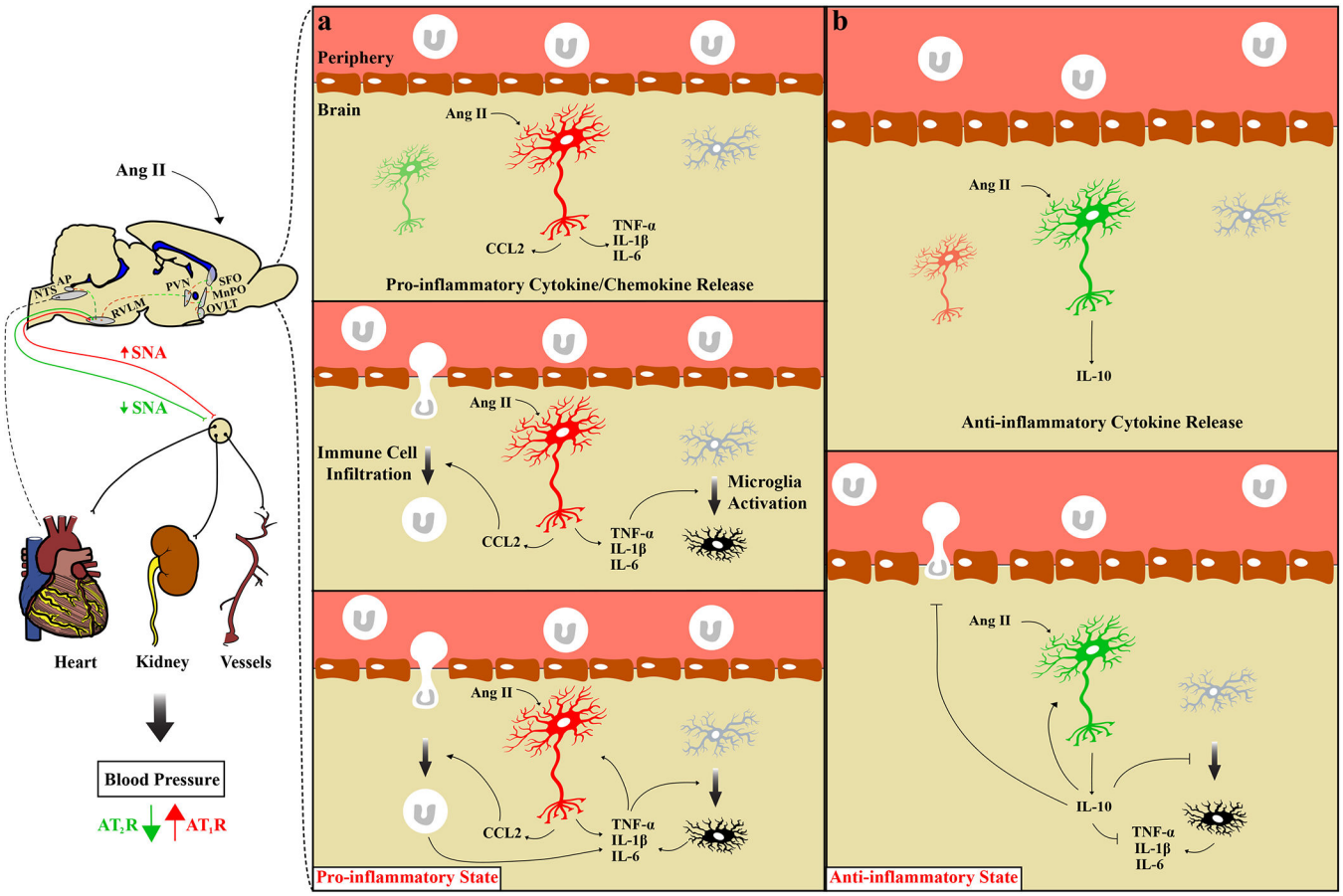

Figure 1. The inflammatory role of brain angiotensin receptors.

A schematic diagram depicting the pro-inflammatory and anti-inflammatory effects of AT_1R of AT_2R respectively. **(a)** Angiotensin II acting on AT_1R expressing neuron (red) mediates the release of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and chemokines (CCL2). Pro-inflammatory cytokines activate microglia, and chemokines facilitate immune cell infiltration (macrophages and T-lymphocytes). Activated microglia and infiltrated immune cells further upregulate the expression of pro-inflammatory cytokines. Pro-inflammatory cytokines can further activate neurons, microglia, and recruit immune cells. This establishes a pro-inflammatory state, which together with the sympatho-excitatory actions of AT_1R, increase sympathetic nerve activity (SNA) and blood pressure. **(b)** Angiotensin II acting on AT_2R expressing neuron (green) mediates the release of anti-inflammatory cytokines (IL-10), which inhibits microglia activation, the recruitment of immune cells and the production of pro-inflammatory cytokines. The anti-inflammatory and the sympatho-inhibitory actions of AT_2R decrease SNA and blood pressure. Pointy head arrow; activation, flat head arrows; inhibition, AP; Area Postrema, MnPO; Median Preoptic Nucleus, NTS; Nucleus of the Solitary Tract, OVLT; Organum Vasculum of the Lamina Terminalis, PVN; Paraventricular Nucleus of the Hypothalamus, SFO; Subfornical Organ.

Table 1.

Expression of AT_1R and AT_2R in the brain cardiovascular centers using transgenic reporter mice.

Expression, distribution, and phenotype of AT_1R/ AT_2R expressing neurons in the cardiovascular centers of the brain, using our recent and novel transgenic reporter mice. % per area represents the percentage of the region of interest occupied by positive cells (AT_1R or AT_2R), and the % Co-loc depicts the colocalization of AT_1R to AT_2R within the region of interest (percentage).

Brain Region	Expression		% per Area		%Co-loc
	AT_1R	AT_2R	AT_1R	AT_2R	
Forebrain					
Subfornical Organ (SFO)	Neuronal soma & Fibers [20]	Fibers [19]	~99 [20]	~1 [20]	~0.2 [20]
Median Preoptic Nucleus (MnPO)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]*a*	~60 [20]	~40 [20]	~2 [20]
Organum Vasculum of the Lamina Terminalis (OVLT)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]	~75 [20]	~25 [20]	~2 [20]
Paraventricular Nucleus of the Hypothalamus (PVN)	Neuronal soma & Fibers [20, 7]*a*	Fibers [19, 11]*b*	~99 [20]	~1 [20]	~0.2 [20]
Hindbrain					
Area Postrema (AP)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]	~40 [20]	~60 [20]	~5 [20]
Nucleus of the Solitary Tract (NTS)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]*b*	~10 [20]	~90 [20]	~3 [20]
Dorsal Motor Nucleus of the Vagus (DMNV)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]*c*	*?*	*?*	*?*
Nucleus Ambiguus (NA)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]*c*	*?*	*?*	*?*
Rostral Ventrolateral Medulla (RVLM)	Neuronal soma & Fibers [20]	Fibers [19]	*?*	*?*	*?*
Caudal Ventrolateral Medulla (CVLM)	Neuronal soma & Fibers [20]	Neuronal soma & Fibers [19]	*?*	*?*	*?*

a; glutamatergic neurons,

b; GABAergic neurons,

c; choline acetyltransferase – containing neurons,

?; not known.

Table 2.

The anti-inflammatory effect of AT_2R.

Different studies utilizing pharmacological AT_2R agonists (C21, CGP42112) or antagonists (PD123319) to demonstrate the anti-inflammatory effects of AT_2R.

Animal	Model	Drug	Effect	Region	Ref
C57BL-6 mice	Experimental Autoimmune Encephalomyelitis	C21 (AT_2R agonist; 0.3 mg/kg; i.p.)	↓ Microglia activation ↓ T-Cell Infiltration	Spinal Cord	[101]
Sprague-Dawley rat	Brain Cell Culture	C21 (AT_2R agonist; 1 μM)	↓ TNF-α, IL-1β, IL-6	N/A	[101]
C57BL-6 mice	LPS - Induced Inflammation	CGP42112 (AT_2R agonist; 1 mg/kg; i.p.)	↓ TNF-α, IL-1β, CCL2 ↑ IL-10	Cerebral Cortex	[102]
Wistar rats	Middle Cerebral Artery Occlusion	C21 (AT_2R agonist; 0.12 mg/kg; oral)	↓ Microglia activation ↓ Macrophage Infiltration	Cerebral Cortex	[33]
Spontaneously Hypertensive rats	Middle Cerebral Artery Occlusion	C21 (AT_2R agonist; 0.14 mg/kg; icv)	↓ Microglia activation ↓ Macrophage Infiltration	Cerebral Cortex	[32]
Wistar rats	DOCA - Salt Induced Hypertension	PD123319 (AT_2R antagonist; 3 μg; icv)	↑ TNF-α, IL-1β ↓ IL-10	PVN	[14]

DOCA; Deoxycorticosterone acetate, icv; intracerebroventricular, i.p; intraperitoneal, LPS; lipopolysaccharide, N/A; not applicable, PVN; paraventricular nucleus of the hypothalamus.