Published in final edited form as:

Trends Endocrinol Metab. 2017 September ; 28(9): 684–693. doi:10.1016/j.tem.2017.06.002.

Centrally-mediated cardiovascular actions of the angiotensin II type 2 receptor

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Abstract

Sustained increases in activity of the sympathetic neural pathways that exit the brain and which increase blood pressure are a major underlying factor in resistant hypertension. Recently available information on the occurrence of angiotensin II type 2 receptors (AT2R) within or adjacent to brain cardiovascular control centers is consistent with findings that stimulation of these receptors lowers blood pressure, particularly during hypertension of neurogenic origin. Up until recently, brain AT2R had not been considered by many to play a role in the central control of blood pressure. Demonstration of these powerful anti-hypertensive effects of brain AT2R opens the door to reconsideration of their role in blood pressure regulation, and their consideration as a novel therapeutic avenue for resistant hypertension.

Keywords

Angiotensin II; Angiotensin Type 2 Receptor; neurogenic hypertension; sympathetic nervous system; vasopressin

Is the AT2R a key to treating resistant hypertension?

Hypertension and the subsequent end-organ damage that occurs as a result of sustained high blood pressure, is one of the major, global health problems and causes of death. The etiology of *essential hypertension*, which is by far the most prevalent form of hypertension, is still unknown. Despite the availability of a number of powerful anti-hypertensive drugs that are able to control blood pressure in the majority of hypertensive patients, between 10–20% of individuals with this disease are resistant to treatment, i.e. targeted blood pressure is not reached with concurrent use of three antihypertensive drugs of different classes, including one diuretic [1–3]. It is commonly thought that a chronic dysregulation of sympathetic outflow, i.e. neurogenic mechanisms based on neuroplasticity and a sensitization of the

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hypertensive response, may be major underlying causes of resistant hypertension [4,5]. Consequently, novel treatment approaches are needed, which target the chronic dysregulation of central blood pressure control.

It is well-recognized that the renin-angiotensin system makes a significant contribution to neurogenic hypertension. The peptide hormone angiotensin II (Ang II) acts via **angiotensin type 1 receptors (AT1R)** (see Glossary) located in various cardiovascular control centers in the CNS to increase blood pressure, effects that are exacerbated in and contribute to sustained hypertension [6]. In contrast, the anti-hypertensive properties of the **angiotensin type 2 receptor (AT2R)** in the CNS have only recently been described. Remarkably, localization and newly identified central effects of AT2R indicate that stimulation of these receptors directly counteracts the dysregulation of sympathetic outflow in neurogenic hypertension. This review summarizes these recent findings on anti-hypertensive, central actions of the AT2R, which – especially in the light of ongoing initiatives for developing AT2R agonists for clinical use – may form the basis for a novel, future approach for the treatment of resistant hypertension.

Overview of the central control of blood pressure and effects of brain AT1R

The physiological regulation of blood pressure (BP) is a complex, orchestrated action of cardiovascular, neural, renal, and endocrine systems, which control BP globally or on a local, tissue-specific level. Blood pressure control by the CNS mainly works through modulation of sympathetic outflow and baroreflex function, which again is a complex process involving several specific brain areas [7]. Key brain areas in this process are the **paraventricular nucleus (PVN)** of the hypothalamus, and the **nucleus of the solitary tract (NTS)** within the dorsal medulla oblongata [7,8]. The PVN receives input concerning volume, osmotic and cardiovascular status via afferents originating within multiple regions within the fore- and hindbrain, and provides adjustments to physiological status via two major efferent systems: activation of magnocellular neurons that project to the posterior pituitary and regulate vasopressin (AVP) secretion, with consequent effects on blood volume and pressure; or activation of pre-sympathetic pathways that project (either directly or via the rostral ventrolateral medulla, RVLM) to the intermediolateral cell column (IML) in the spinal cord for alterations in sympathetic outflow [9]. The NTS is the primary area for baroreflex control via the brain (Figure 1A) [8]. It receives afferent signals from peripheral baroreceptors, located within the aortic arch and carotid sinus, and from various brain areas, processes the incoming signals, and elicits modifications of the excitatory, glutamatergic signals to the caudal ventrolateral medulla (CVLM). These signals determine the intensity of inhibitory, GABAergic signaling from the CVLM to the RVLM. The RVLM eventually determines the ultimate firing rate of sympathetic efferent nerves, which project to key organs involved in BP control such as kidney, heart and blood vessels [8]. Collectively, in a normotensive state, this neuronal control network (NTS => CVLM => RVLM) results in a tonic inhibitory effect on sympathetic outflow. Also of note is that the NTS contains a population of **GABA neurons** that exert an inhibitory influence over the NTS-CVLM glutamatergic pathways (Figure 1A); consistent with this, increasing the level of GABA within the NTS increases blood pressure [10].

Another brain area projecting to the RVLM and influencing sympathetic outflow is the locus coeruleus (LC). The LC is the preeminent noradrenergic nucleus and source of norepinephrine in the brain. Among various other functions, the LC mediates the autonomic response to stressful stimuli [11].

Ang II exerts well-established effects via its AT1R at brain cardiovascular control centers to increase blood pressure [8,12]. This Ang II/AT1R action is mediated through sympathoexcitation and AVP secretion [8,13], and is amplified in and contributes to neurogenic hypertension [6]. In contrast to these actions of brain AT1R, an increasing body of evidence now indicates that brain AT2R, until recently not considered relevant to cardiovascular control, exerts depressor and anti-hypertensive actions, and restorative effects on baroreflex function. These effects are the focus of this article.

Localization of angiotensin receptors in the brain

Since the discovery of the two main types of angiotensin receptors, AT1R and AT2R, there have been attempts to determine their localization in the brain in order to obtain an indication of what their physiological actions in the CNS may be (see Text Box 1). Early studies using radioligand autoradiography and immunostaining [14–16], yielded some initial, important insights into receptor localization, but also had problems such as non-selectivity of antibodies in the case of immunohistochemistry and limited resolution in the case of autoradiography, which did not allow determination of receptor expression at the cellular level. More recently, modern techniques of fluorescence *in situ* hybridization and the development of transgenic mouse models such as AT1R- and AT2R-reporter mice (**AT1aR-tdTomato mice**, derived by breeding AT1a-Cre mice with stop-flox-tdTomato mice, in which all cells containing AT1aR fluoresce red; **AT2R-eGFP-BAC-transgenic mice** in which the fluorophore eGFP is under the control of the AT2R promoter), have allowed new and more detailed insights into angiotensin receptor localization in the brain [17,18]. Recent studies using these approaches have revealed that not only AT1R, but also AT2R, are localized in or near cardiovascular control centers. For example, cell bodies of AT2R-containing neurons are present in the NTS (Figure 2), the dorsal motor nucleus of the vagus (DMNV) and the area postrema, whereas the RVLM contains only fibers/terminals of AT2R neurons [17]. All of these nuclei have an impact on blood pressure control by modifying sympathetic and parasympathetic activity [8]. Moreover, AT2R containing neurons are not present within the PVN; however, there are AT2R neurons which surround the PVN (Figure 2), and both these peri-PVN AT2R neurons and AT2R neurons in the median preoptic nucleus extend processes that terminate within the PVN, where they synapse with AVP magnocellular neurons and pre-autonomic neuron cell bodies [17,19].

Text Box 1

Antagonism between AT2R and AT1R in the brain

Brain AT2R and AT1R exert opposite effects on blood pressure. Generally, it has been proposed that these receptors counteract each other by dimerization and by signaling crosstalk. Thus, the nature of the antagonistic or opposite effects of brain AT2R- and AT1R on blood pressure should be considered, i.e. do they involve direct effects on the

same cells or are these effects on the same or distinct neural circuits? Considering that the majority of AT1R and AT2R are located on different neurons at brain cardiovascular control centers (for example, only ~9% co-localization in the NTS), it is likely that the opposite effects of AT2R and AT1R on blood pressure do not involve a direct antagonistic crosstalk on the same neurons; although, this possibility cannot be entirely excluded as yet. More likely is that the AT1R and AT2R can separately influence the neural circuits that control sympathetic outflow and thereby blood pressure. For example, one scenario is that the AT2R neuron terminals, which are adjacent to the pre-autonomic neurons in the PVN, project to and inhibit the activity of sympathoexcitatory neurons, which get excited upon stimulation of presynaptic AT1R.

The exact functional interactions between brain AT2R and AT1R in controlling blood pressure require more study, in particular under hypertensive conditions, where the levels and cellular location of these receptors may differ from the normotensive state.

Interestingly, when looking at AT2R receptor expression on a cellular level using the novel transgenic mouse strains, it became clear that – at least in adult mice – the only CNS cell type expressing AT2R are neurons [17]. This is in contrast to various studies reporting effects of AT2R stimulation in astrocytes or microglia *in vitro* [20,21]. However, astrocytes and microglia for cell culture are isolated from fetal or newborn rats or mice, in which AT2R, which generally have a higher expression level in fetal than in adult life, may still be present, before they become significantly less in early post-birth life.

Surprisingly, co-localization studies using the newly generated AT1R and AT2R reporter mice revealed that only a small proportion of neurons co-express both, receptors [17]. This is surprising inasmuch as it has been proposed that AT1R and AT2R counteract each other by dimerization and by signaling crosstalk [22,23], which, however, would require that both receptors are localized on one and the same cell. Thus, it is likely that any opposite or counteracting effects of these receptors in the brain are most likely due to actions on separate pathways/neuron systems. Moreover, co-localization studies revealed that AT2R are mainly situated on GABAergic neurons, i.e. neurons with an inhibitory influence on neuronal signal transmission [17]. This observation together with the localization of the receptor in cardiovascular control centers and the PVN might suggest an inhibitory role of the AT2R on AVP synthesis and blood pressure.

AT2-receptors and central effects on blood pressure

A blood pressure lowering effect mediated by central AT2R has recently been shown by several groups using normotensive rats [24–26] and in models of genetic hypertension (spontaneously hypertensive rats/SHR) [24], angiotensin II induced hypertension [26], (DOCA)/NaCl-induced hypertension [27] and 2-kidney 1-clip renovascular hypertension [28]. In conscious animals, this effect could be achieved either by prior over expression of AT2R in certain brain areas (NTS, RVLM) via microinjection of recombinant adeno- or adeno-associated viruses encoding AT2R [26,28], or by chronic intracerebroventricular (icv) infusion of an AT2R agonist over an extended period of time (7 days to 4 weeks) [24,27,29]. It is further noteworthy that the anti-hypertensive effect of *chronic* central AT2R stimulation

is powerful and significantly attenuated the development of hypertension in SHR and in DOCA/NaCl-treated animals [24,27]. However, the effect *chronic* icv infusion of AT2R agonists was weaker or absent in normotensive animals [24,29] or hypotensive rats with heart failure [25], resembling what is seen with other antihypertensive drugs and indicating that the more the mean arterial pressure deviates from the physiological set point, the stronger is the antihypertensive effect of central AT2R stimulation. Short-term icv infusion (~ hours) of an AT2R agonist into anesthetized normotensive rats also failed to decrease blood pressure [30]. One report also indicates that acute microinjection of an AT2R agonist into the AT2R-rich IML of anesthetized normotensive rats resulted in a significant fall in blood pressure [31]. The findings which demonstrate that the blood pressure lowering effects of icv-applied AT2R agonists require chronic infusion, whereas a profound effect can be obtained with direct tissue (IML) application, might suggest that these actions are occurring via different mechanisms. The blood pressure lowering effect of AT2R stimulation was also attenuated by ovariectomy in female rats, which was most likely due to a decrease in AT2R expression in the PVN (and potentially other cardiovascular control areas) in ovariectomized rats [27]. Interestingly, Dai et al. report that female rats have higher AT2R mRNA and protein expression in the PVN when compared with male rats; they further suggest that these higher AT2R levels elicit endogenous protection from the development of hypertension – in this study DOCA/NaCl-induced hypertension – only in females but not in males [32]. One consideration about this study is that the AT2R which were measured are likely associated with the peri-PVN AT2R neurons, as AT2R-containing neurons are not localized within the PVN [17].

In contrast to the strong centrally-mediated anti-hypertensive effect, peripheral (i.p., oral) application of AT2R agonists has usually been reported to have no effect on normal or elevated blood pressure [33]. There are only a few exceptions from this lack of effect, namely studies with intrarenal application of the AT2R agonist C21 in Ang II induced hypertension [34,35], and studies in Zucker Obese Fatty rats [36,37].

AT2R and the regulation of sympathetic outflow

As elaborated on in previous sections, one of the main mechanisms by which the CNS controls systemic blood pressure, is by modification of sympathetic outflow [8]. With regard to the CNS-mediated anti-hypertensive effect of the AT2R, it has indeed been reported by various groups, that central AT2R stimulation results in an inhibition of sympathetic outflow, which coincides with the anti-hypertensive effect. For example, Brouwers et al. [24] and Gao et al. [25] reported an inhibition of renal sympathetic nerve activity (RSNA) and a decrease in norepinephrine levels in urine after icv infusion of C21 over 7 days in normotensive and hypertensive rats. Furthermore, direct microinjection of an AT2R agonist into the IML in normotensive rats produced sympathoinhibition [31]. Central AT2R stimulation by icv infusion was also able to reduce sympathetic outflow in heart failure [25], which is a state of profound sympathoexcitation [38].

In this context, AT2R in the main cardiovascular control centers seem to play an important role in the sympathoinhibitory effect, because viral overexpression of the AT2R in the NTS and the RVLM both led to an inhibition of sympathetic outflow and blood pressure [26,28].

The mechanisms by which AT2R stimulation attenuates sympathetic outflow are only partly understood, but seem to be multimodal and involve a decrease in norepinephrine secretion from the locus coeruleus to the hypothalamus [11], stimulatory effects on neuronal potassium current resulting in hyperpolarization and reduced firing rates [39,40], and an intact central NO pathway [24].

A key finding, which points to a major mechanism of AT2R-mediated blood pressure control, is the predominant localization of brain AT2R on GABAergic neurons. Thus, stimulation of AT2R on GABAergic neurons may enhance the inhibitory effects of GABA signaling on excitatory key signaling pathways controlling sympathetic nerve activity; for example, the GABAergic neurons originating from the CVLM and extending into the RVLM, where they synapse onto and inhibit sympathetic neurons [8] (Figure 1A). AT2R positive neurites extending into the RVLM have indeed been found in the AT2R-eGFP-BAC-transgenic reporter-mouse [17], but both the origin of these fibers and their functional roles still needs to be identified. However, as will be described in more detail in the following section, experimental evidence has already been provided for an AT2R induced enhancement of the inhibitory effect of GABAergic neurons on AVP release in the PVN (see also Text Box 2) [19].

Text Box 2

AT2R and GABA interactions

GABA is widely known as an inhibitory transmitter in the brain; GABA neurons are widespread, and GABA can exert its functional effects through GABA_A- or GABA_B receptors. Evidence to date indicates that a majority of AT2R in the brain, including those at or near to cardiovascular control centers, are located on GABA neurons. The data available thus far suggest that there are at least two ways in which AT2R can influence the activity or function of GABA neurons and influence cardiovascular and hormonal function, as depicted in Figure 3, Key Figure. In the first instance (Figure 3, panel A), AT2R located on GABA neurons within the NTS act to depress GABA synthesis (and ultimately release), reducing the tonic inhibition of blood pressure lowering baroreflex pathways provided by these GABA neurons; the result of this AT2R action would be to lower blood pressure further.

In the second instance, AT2R-containing GABA neurons in the peri-PVN region, which extend to and synapse with magnocellular AVP neurons, are excited by AT2R agonist treatment; furthermore, AT2R agonist treatment of these neurons increased the frequency of GABA_A receptor-mediated inhibitory postsynaptic currents in AVP neurons, depressing their activity and ultimately AVP secretion (Figure 3, Panel B).

In contrast, preliminary data from our group suggest that central AT2R stimulation can also result in an *attenuation* of GABAergic signaling, in the NTS, by inhibition of GABA synthesis at this site [41]. If this turns out to be depression of the NTS GABA neurons that provide a tonic inhibitory influence over the NTS-CVLM glutamatergic pathways (Figure. 1B), then the end result would be a lowering of sympathetic outflow and blood pressure.

AT2R and the baroreflex function

Neurogenic hypertension is not only characterized by sustained sympathoexcitation, but also by impaired baroreflex function [5]. As noted above, and shown in Figure 2, there is a high density of AT2R in the NTS, an area that has a major role in controlling baroreflex function [8,42]. A number of studies have demonstrated that selective activation of AT2R can exert restorative effects on baroreflex function, particularly in disease-states. For example, icv-infused C21 improves baroreflex sensitivity in heart-failure rats [25], and also in SHR [24]. In a different approach, chronic AAV2-mediated over expression of AT2R within the NTS restored baroreflex sensitivity in the two kidney-1 clip- and SHR models of neurogenic hypertension [28,43]. Collectively, these studies suggest that AT2R, particularly those in the NTS, serve to improve baroreflex function in concert with sympathoinhibition; these effects are opposite to the sympathoexcitation and impairment of baroreflex function elicited through over activity of AT1R.

AT2R and regulation of vasopressin release

The central and peripheral RAS have a major impact on another major cardiovascular hormone, which is vasopressin (AVP). Approximately 10 years ago, co-localization studies using electron microscopic dual immunolabeling of the AT2R and AVP indicated for the first time that there is a co-localization of AT2R and AVP containing neurons in the PVN [44]. Since the PVN is the main area of AVP synthesis, this observation pointed to a role of the AT2R in the control of AVP generation. Interestingly, the authors of this initial study reported localization of AT2R mainly on neuronal processes in the PVN, but not or only very rarely on soma [44]. The same pattern was found by our group using the AT2R-eGFP-BAC-transgenic reporter-mouse: AT2R-positive, fluorescent green staining was not detectable on the soma of AVP neurons within the PVN, but on processes and nerve terminals arising from AT2R-positive cell bodies in the peri-PVN area, which extended into the PVN and synapsed onto AVP neurons [17,19]. The majority of these AT2R-positive neurons turned out to be GABAergic [17,19], and consequently, selective stimulation of these inhibitory neurons by the AT2R agonist C21 led to reduced activity of AVP neurons within the PVN, thus reducing AVP release and eventually decreasing AVP plasma levels.

Importantly, this was the first time that inhibitory, GABAergic neurotransmission was identified to be an essential mechanism, by which central AT2R stimulation exerts its primarily inhibitory action on CNS effects.

Concluding remarks and future perspectives

As reviewed in the above paragraphs, latest research using novel techniques such as GFP-expressing reporter mice, which enable the localization of angiotensin AT2R with high resolution and at the cellular level, revealed that in contrast to prior knowledge this receptor is expressed on neurons in and around major cerebral cardiovascular control centers and on neurons synapsing with AVP releasing neurons in the PVN. The majority of these AT2R positive neurons are GABAergic. Several independent studies simultaneously revealed a

blood pressure lowering effect and a reduction in sympathetic outflow when the CNS was chronically exposed to AT2R-stimulation or –overexpression.

It will be a major future task for researchers interested in the central actions of AT2R to prove or disprove a link between the central, physiological effects of AT2R and GABAergic signaling in the CNS. Moreover, the nature of the effect of AT2R stimulation on GABAergic neurons needs to be identified for every single, relevant brain area, since according to current, still very limited and partly preliminary data, such effects can range from stimulation of GABAergic nerve activity to inhibitory effects on GABA synthesis.

Importantly, the latest findings about signaling and function of AT2R in the brain strongly suggest that central AT2R stimulation directly counteracts a main patho-mechanism of neurogenic hypertension, which is sympathetic overdrive (see Text Box 3). It does so by modifying the activity of GABAergic nerves, which directly synapse with main neuronal pathways of cardiovascular and sympathetic outflow control.

Text Box 3

AT2R agonists as anti-hypertensive agents?

The general lack of effect of AT2R agonists to lower blood pressure when administered systemically, except under certain conditions, is in contrast to their significant depressor effects when infused into the brain. Based on the available data, it is reasonable to conclude that the AT2R within or near brain cardiovascular control centers constitute an endogenous blood pressure-lowering mechanism that might be taken advantage of in the clinical situation by using a selective and potent AT2R agonist to elicit anti-hypertensive effects. Thus, developing an AT2R agonist to target brain AT2R might represent a novel anti-hypertensive therapeutic strategy. However, a major problem to be overcome – apart from carefully excluding any unwanted, central effects of AT2R stimulation – would be efficient delivery of AT2R agonists into the brain, i.e. a route of application ensuring sufficient tissue levels in the cardiovascular control centers. AT2R agonists currently in drug development are blood brain barrier (BBB) impermeable. Potential solutions for achieving BBB penetrance may be chemical modifications of the available AT2R agonists in order to increase lipophilicity or to avoid that these agonists are substrate for efflux pumps such as p-glycoprotein. Other options may comprise the use of receptor-mediated transcytosis, embedding of drugs into nanoparticles or other specific carriers, which penetrate the BBB, or blockade of efflux carriers. Finally, intranasal application of AT2R agonists may be considered, whereby the drug would be transported from the upper nasal cavities to the forebrain (via olfactory pathways) and the brain stem (via trigeminal nerves). The lately developed method of high-intensity focused ultrasound for making the BBB permeable seems too invasive for an indication such as hypertension, which requires a long-term, regular treatment.

Consequently, and in light of the potential future clinical availability of selective AT2R agonists (two AT2R agonists, C21 and MP-157, are in clinical development), central AT2R

stimulation should be considered and furthermore, be tested as a future therapeutic approach for the treatment of neurogenic, resistant hypertension.

Glossary

Angiotensin II type 1 receptor (AT1R)

receptor mediating the “classical” effects of angiotensin II such as vasoconstriction, Na⁺/H₂O-retention, stimulation of vasopressin and aldosterone release

Angiotensin II type 2 receptor (AT2R)

receptor mediating the “protective” effects of angiotensin II such as vasodilation, anti-inflammation, inhibition of vasopressin (AVP) secretion.

AT1aR-tdTomato mice

derived by breeding AT1a-Cre mice with stop-flox-tdTomato mice, in which all cells containing AT1aR fluoresce red

AT2R-eGFP-BAC-transgenic mice

mice in which the fluorophore eGFP is under the control of the AT2R promoter and all cells containing AT2R fluoresce green.

GABAergic neurons

neurons, in which the neurotransmitter is gamma-aminobutyric acid GABA; typically, such neurons synapse with excitatory (e.g. glutamatergic) neurons thus reducing their excitability.

Nucleus of the solitary tract (NTS)

It is within the dorsal medulla oblongata and has a major role in controlling baroreflex function and sympathetic outflow.

Paraventricular nucleus of the hypothalamus (PVN)

It contains pre-autonomic nerves and also magnocellular AVP neurons. It is a major brain area for collecting and integrating information concerning body fluid and cardiovascular status, and for regulating sympathetic outflow and AVP secretion.

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Trends Box

- The angiotensin type 2 receptor (AT2R) is the main receptor of the protective arm of the renin-angiotensin system.
- Latest research applying novel transgenic animal models revealed that AT2R are localized within and around central cardiovascular control centers.
- AT2R in the CNS are exclusively located on neurons, the majority of which are GABAergic.
- Stimulation of central AT2R results in inhibition of sympathetic outflow and lowering of blood pressure, which is most likely a result of a modification of GABAergic modulation of cardiovascular control and sympathetic drive.
- AT2R-containing GABA neurons project to the paraventricular nucleus and regulate the secretion of vasopressin.

Outstanding questions

- What is the normal physiological role of the endogenous, AT2R related brain depressor mechanism?
- If brain AT2R constitute an endogenous brain anti-hypertensive mechanism, why is this mechanism unable to prevent the development of neurogenic hypertension?
- Is a modification of GABAergic signaling the main mechanism through which AT2R exert their central anti-hypertensive effect?
- Is there a direct crosstalk between central AT2R and AT1R in the control of blood pressure, or are both receptors acting in a purely independent way?
- Do astrocytes and/or microglia play any role in the central anti-hypertensive effect of AT2R agonists?
- How can AT2R agonists, which are blood brain barrier impermeable, be efficiently and non-invasively delivered to brain cardiovascular control centers?
- Can the anti-hypertensive effect of brain AT2R stimulation as observed in preclinical hypertension models be translated into the human situation?

A.

Without AT2R Stimulation

B.

With AT2R Stimulation

Figure 1. Hypotheses for modification of GABAergic signaling in the NTS by AT2R stimulation
 AT2R stimulation is able to modify the baroreflex by altering GABAergic signaling in the NTS.

A: When blood pressure rises, baroreceptors are activated and signal into the NTS (1). The NTS receives input from various brain areas including the baro-afferents and processes these signals resulting into an excitatory signal into the CVLM (2). In the CVLM, this excitatory signal leads to activation of GABAergic neurons, which extend to the RVLM and eventually inhibit sympathetic outflow (3). The stronger the excitatory signal from baroreceptors/NTS, the stronger the GABAergic inhibition of sympathetic outflow (4). The GABAergic nerve fibers within the NTS can modify the baroreflex by inhibition of the excitatory signal from NTS to CVLM, which results in an attenuation of the inhibition of sympathetic outflow.

B: AT2R stimulation in the NTS inhibits synthesis of GABA, which depresses the GABAergic inhibition of the baroreflex and eventually leads to a stronger diminution of sympathetic outflow and a greater lowering of blood pressure.

Figure 2. Localization of AT2R within or near brain cardiovascular control centers

Panels A and C: Low power representative coronal sections through the forebrain, including the PVN (A) and medulla, including the NTS (C) of the AT2R-eGFP transgenic reporter mouse [17]. Location of AT2R containing cells is depicted by green eGFP fluorescence. Scale bars are 1mm.

Panels B and D: High power fluorescence micrographs showing the presence of AT2R-containing cells (green) surrounding the PVN (B) and within the NTS (D). Scale bars are 100 μ m and 200 μ m for panels B and D respectively.

Key: PVN = Paraventricular Nucleus of the Hypothalamus; NTS = Nucleus of the Solitary Tract; AP = Area Postrema; CC = Central Canal; DMX = Dorsal Motor Nucleus of the Vagus; HG = Hypoglossal Nucleus.

Figure 3, Key Figure.

Hypotheses for the differential influences of AT2R on GABA neuron signaling in cardiovascular and hormonal control

The two panels in this figure depict the potential outcomes of selective activation of AT2R within or near CNS cardiovascular control centers.

(A). Activation of AT2R located on GABA neurons within the NTS depresses GABA synthesis (and ultimately release), and reduces the tonic inhibition of blood pressure lowering baroreflex pathways provided by these GABA neurons. Result: further lowering of blood pressure.

(B). Activation of the AT2R-containing GABA neurons in the peri-PVN region that synapse with magnocellular AVP neurons in the PVN leads to increased secretion of GABA. Consequently, the activity on AVP-containing cells is depressed. Result: decreased AVP secretion.