AT2 Receptor Signaling and Sympathetic Regulation

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Abstract

There is a growing consensus that the balance between Angiotensin Type 1 (AT1R) and Angiotensin Type 2 (AT2R) signaling in many tissues may determine the magnitude and, in some cases the direction, of the biological response. Sympatho-excitation in cardiovascular diseases is mediated by a variety of factors and is, in part, dependent on Angiotensin II signaling in the central nervous system. Recent data have provided evidence that the AT2R can modulate sympatho-excitation in animals with hypertension and heart failure. The evidence for this concept is reviewed and a model is put forward to support the rationale that therapeutic targeting of the central AT2R may be beneficial in the setting of chronic heart failure.

Introduction

The renin angiotensin system (RAS) impacts a variety of important biological functions that are critical to circulatory and fluid volume homeostasis. It has been long accepted that the octapeptide Angiotensin II (Ang II) possesses potent effects in the central nervous system that regulate and modulate thirst, salt appetite, vasopressin release and sympathetic nerve activity. In this way strong activation of the central RAS can contribute to the pathogenesis of hypertension and other sympatho-excitatory states such as heart failure. There have been many excellent reviews on this topic [1**–5] and we will not attempt to summarize all the evidence for the central sympatho-excitatory effects of Ang II. Rather, this review will focus on an emerging area of central Ang II signaling through the Angiotensin Type 2 receptor (AT2R).

Angiotensin II Receptor Subtypes in the CNS

The central nervous system is well endowed with the two main receptor subtypes, AT1R and AT2R. These receptors are ubiquitously distributed in the brain and spinal cord [6–8] and located on neural, glial and vascular elements [9;10]. While Angiotensin II receptors are expressed throughout the brain, there appears to be a high density in those areas of the hypothalamus and medulla that regulate sympathetic outflow, arterial baroreflex function and thereby blood pressure [6;11;12]. This is especially relevant in areas that have no blood brain barrier and send projections to nuclei in the hypothalamus and medulla; the so called circumventricular organs [13]. In addition, there is evidence that an AT3R exists [6;14] and a small amount of evidence suggesting the possibility of a non AT1, AT2 or AT3 receptor signaling pathway [6;15].

In the central nervous system the downstream signaling pathways for Ang II are much the same as they are in other tissues. Both AT1 and AT2 receptors are G-protein coupled and signal through Gq and Gi, respectively [16]. Because the AT2R increases nitric oxide (NO) release [17;18] and facilitates neuronal potassium current [19], activation of this receptor should evoke sympatho-inhibition. This notion has been difficult to confirm, especially in disease states, because of the relative predominance of the AT1R and its sympatho-excitatory effects.

The prevailing dogma is that the AT2R subtype in the brain is predominant in the fetus, while the AT1R subtype is predominant in adults. This is based primarily on studies using autoradiography [20;21*], quantitative autoradiography [22], and *in situ* hybridization [23] techniques. Unfortunately, there are no data at the protein level to confirm or refute this idea.

A recent study from our laboratory revealed a different Angiotensin receptor profile in both rats and mice during development which contrasts that currently based on the above studies. Using Western Blot analysis, we clearly demonstrated that, in brainstem, liver, and kidney, adult rats exhibit significantly higher AT2R, but significantly lower AT1R, protein expression compared to fetal or neonatal rats [24**]. Moreover, in the developmental mice, we got the same results as in rats. Figure 1 shows the time course of AT2R and AT1R protein expression in the brainstem from fetus to 6 week old mice. This figure clearly shows a gradual increase in AT2R expression in the brainstem during progression from fetal to adult life. On the other hand, expression of the AT1R appears to gradually decrease during maturation. It is not clear at which point in time this reversal in receptor expression occurs. However, 4 week old mice exhibit the same AT2R expression as do older mice, suggesting developmental changes in AT2R expression are complete in the mice at around one month. For the AT1R, stable expression appears at the 3 week time period, one week earlier than AT2R.

Based on our rodent data, it is our belief that the AT1R is always the dominant receptor subtype at all developmental stages, with higher expression in the fetus and neonate and lower expression in adulthood. On the other hand, the AT2R is expressed in a lower degree at first with an expression pattern which increases following birth into adulthood. The ratio of AT1R to AT2R protein therefore, is higher in early development compared to adulthood.

The reason for the differences in ATR protein expression versus the previous autoradiographical data is not completely clear. The autoradiography is a classical pharmacological method to detect receptor-ligand binding, which is a highly sensitive technique but its validity largely depends on the specificity of agonist and antagonist employed. In the previous autoradiographical study [20;21] whole animal binding was examined rather than select brain regions. Furthermore, these results were based primarily on changes in binding in response to AT1R or AT2R antagonists whereas we used specific antibodies to evaluate protein expression in various brain areas. Nevertheless additional work needs to be done in order to determine the mechanism for these differences.

If our biochemistry data are translated into functional significance, they suggest that activation of the AT2R may be a therapeutic strategy to limit the effects of AT1R stimulation in the adult in several diseases that are characterized by sympatho-excitation, such as chronic heart failure, hypertension, and diabetes by virtue of the fact that AT2R stimulation opposes activation of the AT1R in sympathetic regulatory areas of the central nervous system. Recent data from this laboratory have shown the functional influence of central AT2R activation on sympathetic outflow in adult rats using the AT2R agonist

CGP123319 [25*] and by AT2R over expression using gene transfer [26], which is summarized in the following section.

Central AT2R and Sympathetic Regulation

Data from mice with AT2R gene deletions suggest the contribution of this receptor to sympatho-inhibition [27;28]. While the AT1R in the central nervous system has been solidly linked to sympatho-excitation, AT2R activation exhibits opposite influences on sympathetic tone. Siragy et al. [29] reported that AT2-null mice had slightly elevated systolic blood pressure compared with that of wild-type control mice. Infusion of a subpressor dose of Ang II in wild-type mice resulted in a pressor response in AT2R gene knock-out mice. Moreover, Li et al. [30] found that intracerebroventricular (icv) injection of Ang II evoked a larger increase in blood pressure in AT2R gene knock-out mice compared to wild type mice. In wild type mice central injection of Ang II plus PD123319 (an AT2R antagonist) initiated a greater pressor response than that induced by Ang II alone. These results strongly imply a potential inhibitory effect of stimulating central AT2Rs on blood pressure, which is likely mediated by sympatho-inhibition.

In a recent study we demonstrated that adenoviral gene transfer of the AT2R gene induced overexpression of AT2R protein in the rostral ventrolateral medulla (RVLM; a primary brainstem nucleus related to the control of sympathetic outflow) suppressed norepinephrine excretion and reduced arterial blood pressure in normal rats [26]. Similar effects were recently described by Li et al. [31]. We further found that in rats with chronic heart failure AT2R expression in the RVLM was significantly lower than in normal rats. This downregulation of AT2R expression contributed to the sympatho-excitation in this syndrome[25]. The regulation of cardiovascular function by AT2Rs in the RVLM was also recently documented by Tedesco and Ally who focused on the exercise pressor reflex [32]. These authors found that bilateral microdialysis of PD123319, the selective AT2R antagonist, into the RVLM augmented the pressor and tachycardia responses to static muscle contraction in anesthetized rats. They further demonstrated that the amplification by PD123319 contributed to the increased glutamate and decreased GABA levels within the RVLM. These data suggest that AT2Rs in the RVLM exhibit their influence on sympathetic outflow via regulating release of classical neurotransmitters locally. In addition, our recent experiments performed in normal rats demonstrated that chronic icv infusion of Compound 21 (C21), a newly created non-peptide AT2R agonist, decreased nocturnal norepinephrine (NE) excretion and blood pressure via a nNOS/NO signaling pathway within PVN and RVLM. Figure 2 shows NE concentration (ug/mL, panel A) and NE excretion (ug/12h, panel B) from day time and night time urine after C21 treatment for 7 days. C21 treated rats exhibited a significantly lower nocturnal NE concentration and excretion compared with control rats, suggesting a central inhibitory influence of C21 on sympathetic outflow.

While it has been demonstrated that overexpression of AT2R in the periphery contributes to hypotensive responses following losartan administration [33], little information is available concerning the role of AT2Rs in the central nervous system on blood pressure or sympathetic regulation. AT2Rs appear to oppose enhanced renal sympathetic neurotransmission in response to stimulation of the AT1R [34]. On the other hand, Nap et al. [35] provide *in vitro* evidence to the contrary. Toney and Porter [36] showed that the AT2R was involved in the central vasopressin response to Ang II in young (4 week old) rats. This notion has recently been substantiated by electron micrographic-double labeling experiments showing the localization of AT2R and vasopressin on neurons and glia in the PVN [37]. While some data from anesthetized rats suggest that AT1R stimulation of RVLM neurons are inhibitory [38], most suggest that this stimulation is excitatory [25;39;40]. New data

from our laboratory using a specific agonist for the AT2R suggests that activation of this pathway may, indeed, modulate excitatory Ang II responses in the RVLM [25].

AT2R and Neuronal Electrophysiology

Data from patch-clamp and extracellular single unit discharge recordings provide evidence for the above-mentioned inhibitory influence of central AT2R activation on sympathetic drive. Kang et al. [19] demonstrated that stimulation of AT2Rs significantly increased neuronal voltage-gated potassium channel current (Ikv) in cultured neurons from newborn rat hypothalamus and brainstem. They further indicated that the third intracellular loop of the AT2R is a key component in the stimulation of neuronal Ikv elicited by activation of this receptor [41]. Martens et al. [42] reported that activation of AT2R by CGP42112 modulates rat hypothalamic and brainstem neuronal whole-cell K^+ current by increasing their open probability. Moreover, further study has demonstrated that, in single neurons, superfusion with Ang II (stimulation of AT1R and AT2R) induced a decrease in Ikv while superfusion of Ang II plus Losartan (stimulation of AT2R) induced an increase in Ikv, and superfusion of Ang II plus losartan and PD123319 resulted in no alterations of Ikv [43]. Consistent with the effects of AT2R stimulation on neuronal potassium current, Matsuura et al. [44] recently demonstrated a AT2R-mediated hyperpolarization and decrease in firing rate in bulbospinal RVLM neurons. Employing intracellular recording using the whole-cell patch-clamp technique and the brain stem-spinal cord preparation from AT1R knockout mice, they found that the resting membrane potential tended to be more negative, and the firing rate tended to be slower in bulbospinal RVLM neurons. They further found that superfusion with Ang II in AT1R KO mice hyperpolarized RVLM bulbospinal neurons and decreased their firing rate. On the other hand, using intracellular recordings, iontophoretic, micropressure and bath perfusion methods, Xiong and Marshall [45;46] demonstrated that Ang II, via stimulating the AT2R, depresses glutamate depolarizations and excitatory postsynaptic potentials in Locus Coeruleus of a brain slice preparation. Interestingly, the AT2R induced neuronal activation of delayed rectifier potassium channels has also been demonstrated to have a neuroprotective effect which is mediated through the AT2R [47]. These data strongly imply that AT2Rs supress neuronal activity via facilitating potassium channel current and decreasing firing rate.

The majority of the neuronal AT2R intracellular signaling pathways are mediated via inhibitory G proteins (Gi) [41;48], even though evidence also exists showing that AT2Rs can couple via a G-protein-independent mechanism [49]. It has been demonstrated in neurons cultured from neonatal rat hypothalamus and brainstem, that inhibitory G proteins, PLA2, and protein phosphatase 2A (PP2A) are involved in the AT2R-dependent increase in K^+ current [50–52]. AT2R activation stimulates PP2A activity [53] and PP2A may directly participate in a dephosphorylation-mediated activation of the K^+ channel. Zu et al. [54] explored the involvement of a series of arachidonic acid (AA) metabolites in the AT2R-evoked increase in the K^+ current in cultured neurons. They demonstrated that the PLA2/AA/12-LO pathway is responsible for the modulation of K^+ currents by AT2R. This activation of the delayed rectifier K^+ current (an outward K^+ current in neuronal cells) will result in hyperpolarization of cellular membranes and suppression of neuronal activity. In preliminary experiments using CATH.a neurons, we demonstrated that C21 treatment significantly increased neuronal I_{Kv}, which was completely abolished by the AT2R antagonist, PD123319, and the NOS inhibitor, L-NAME (figure 3).

Central AT2R and Therapeutic Strategy

Many cardiovascular diseases, including chronic heart failure, essential hypertension, and diabetes, are characterized by heightened sympathetic tone. This excessive sympathetic

outflow contributes to a variety of deleterious effects on the cardiovascular system, such as increased myocardial oxygen consumption and decreased blood flow to peripheral organs. Sympatho-excitation, therefore is an important therapeutic target and suppression of sympathetic outflow has become mainstream therapy for these diseases. Given the down regulated central AT2R expression in both chronic heart failure [25] and hypertension [55] and its role in the sympatho-excitation of these diseases, up regulation of AT2R expression in sympathetic regulatory areas of the brain by gene transfer or other methods, may have therapeutic potential in these diseases. As we have previously shown [26] viral vector-induced overexpression of the AT2R in the RVLM is associated with a reduction in blood pressure and urinary norepinephrine excretion. It will be relevant to determine the effects of AT2R overexpression in the heart failure state. In addition, C21 may be a valuable sympatho-inhibitory substance capable of activating central AT2Rs. Even though most reports have demonstrated its beneficial influence in spontaneously hypertensive rats [15] and rats with myocardial infarctions [56], our preliminary data suggest the inhibitory effects of C21 on sympathetic outflow is mediated by central mechanisms.

Conclusion and perspective

In this review we have concentrated on central AT2Rs and their potential involvement in sympathetic regulation. Although expression and functional significance of AT2Rs is still controversial new data have demonstrated a higher protein expression level of this receptor in mature rodent compared to that in the fetus and early neonate. Consistent with the Western blot data, we also documented an inhibitory influence of central AT2Rs on sympathetic tone in normal adult rats. In addition, the down regulated AT2R expression and signaling in the RVLM have been shown to contribute to the sympatho-excitation in chronic heart failure. These findings raise the possibility of a potential therapeutic strategy in diseases characterized by sympatho-excitation. The newly created non-peptide AT2R agonist, Compound 21 may potentially provide therapeutic efficacy. Figure 4 summarizes a possible therapeutic strategy that targets sympatho-excitation through stimulation of central AT2Rs or by overexpression of AT2R protein.

Acknowledgments

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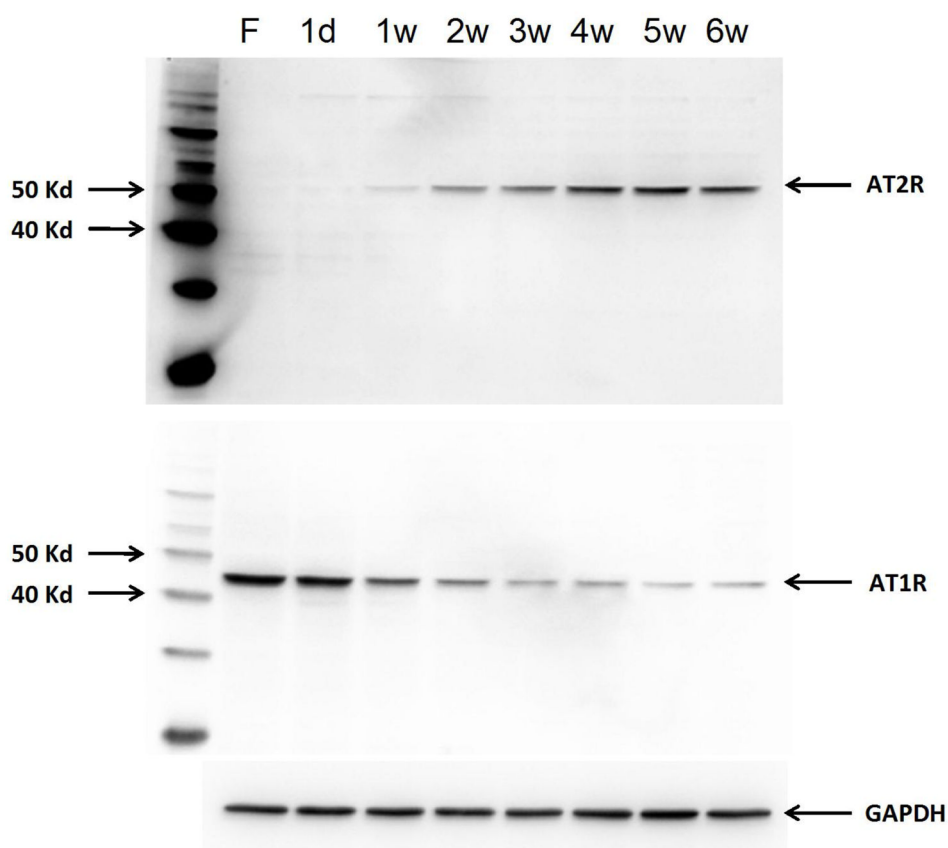
Figure 1.
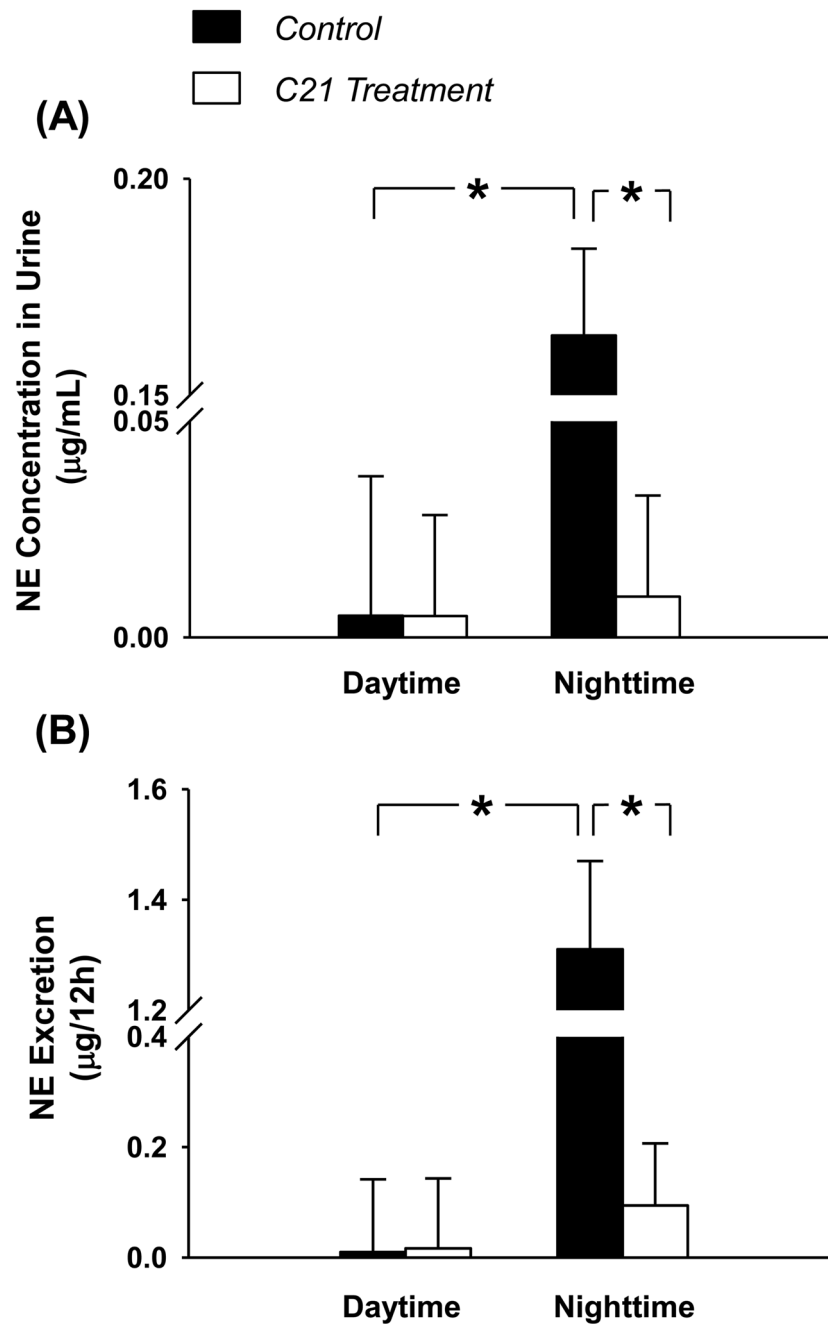Developmental changes in AT2R and AT1R protein expression in brainstem of mice. F: Fetus, d: day, w: week(s)

Figure 2.
NE concentration (panel A) and NE excretion (panel B) in day time and night time urine after icv infusion of C21 for 7 days. *P < 0.05, n = 8 for the control group and 10 for the C21 treated group.
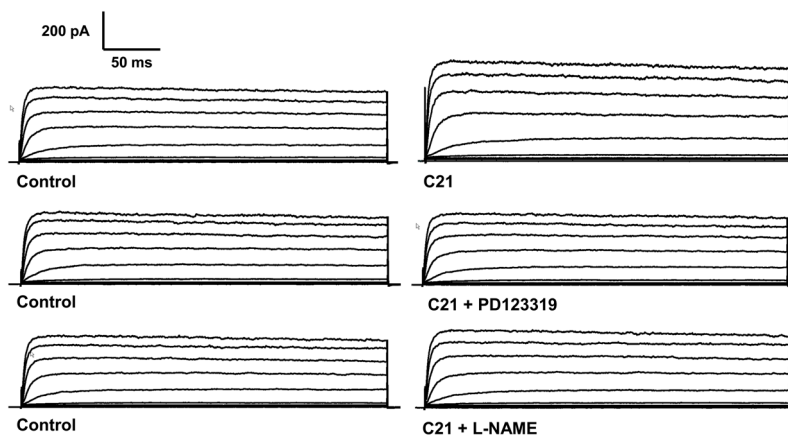
Figure 3.
Current tracings of I_{Kv} in CATH.a cells (a neuronal cell line) measured by whole cell patch clamp. C21: AT2R agonist; PD123319: AT2R antagonist; L-NAME: NOS blocker.
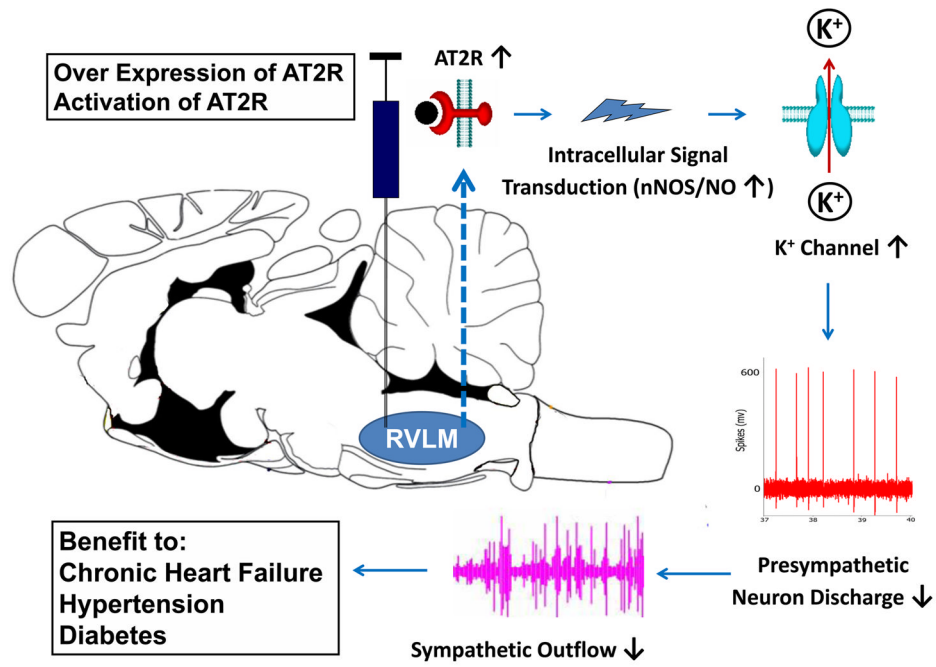
Figure 4.
Outline of therapeutic strategy by which activation or over expression of central AT2Rs may reduce sympatho-excitation in cardiovascular diseases such as heart failure and hypertension. RVLM: rostral ventrolateral medulla. (Modified from Zimmerman MC and Davisson RL [57]).