Regulation of central angiotensin type 1 receptors and sympathetic outflow in heart failure

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Zucker IH, Schultz HD, Patel KP, Wang W, Gao L. Regulation of central angiotensin type 1 receptors and sympathetic outflow in heart failure. Am J Physiol Heart Circ Physiol 297: H1557–H1566, 2009. First published August 28, 2009; doi:10.1152/ajpheart.00073.2009.—Angiotensin type 1 receptors (AT1Rs) play a critical role in a variety of physiological functions and pathophysiological states. They have been strongly implicated in the modulation of sympathetic outflow in the brain. An understanding of the mechanisms by which AT1Rs are regulated in a variety of disease states that are characterized by sympathoexcitation is pivotal in development of new strategies for the treatment of these disorders. This review concentrates on several aspects of AT1R regulation in the setting of chronic heart failure (CHF). There is now good evidence that AT1R expression in neurons is mediated by activation of the transcription factor activator protein 1 (AP-1). This transcription factor and its component proteins are upregulated in the rostral ventrolateral medulla of animals with CHF. Because the increase in AT1R expression and transcription factor activation can be blocked by the AT1R antagonist losartan, a positive feedback mechanism of AT1R expression in CHF is suggested. Oxidative stress has also been implicated in the regulation of receptor expression. Recent data suggest that the newly discovered catabolic enzyme angiotensin-converting enzyme 2 (ACE2) may play a role in the modulation of AT1R expression by altering the balance between the octapeptide ANG II and ANG-(1–7). Finally, exercise training reduces both central oxidative stress and AT1R expression in animals with CHF. These data strongly suggest that multiple central and peripheral influences dynamically alter AT1R expression in CHF.

sympathetic nerve activity; neuronal signaling; oxidative stress

SINCE THE ORIGINAL DISCOVERY of a pressor substance emanating from the kidney by Tigerstedt and Bergman in 1898 (5, 141), the role of angiotensin II (ANG II) in the regulation of arterial pressure has been intensively investigated. This primitive octapeptide has been implicated in a multitude of biological processes in health and disease. The pivotal role that ANG II plays in salt, water, and cardiovascular homeostasis and in blood vessel and cardiac remodeling has catapulted it as an important target for therapy in a variety of disease states (25, 95, 105, 106). ANG II and its receptors are important determinants of autonomic tone (121). ANG II receptors are ubiquitous in both the nervous and cardiovascular systems (6, 64, 65, 99, 107, 138, 142, 158), and ANG II is important in the long-term regulation of arterial blood pressure, as summarized in several excellent reviews (4, 13, 121). An important question remains as to the relative importance of the neural effects of ANG II versus its more established effects on salt and water balance (89, 90) and on the vasculature (60, 145).

Over the past several years the complexity of the brain renin-angiotensin system (RAS) has grown with the addition of various components and the discovery of new, potentially important components and their compartmentalization (63). The recent discovery of a catabolic enzyme that cleaves one amino acid from ANG II to form ANG-(1–7) is providing new details in the regulation of ANG II at the tissue level (46, 76). The balance between angiotensin-converting enzyme (ACE) and its homolog ACE2 may be an important determinant of sympathoexcitation in the setting of chronic heart failure (CHF).

There is no question that the integrated, long-term systemic response to activation of the RAS involves renal, cardiovascular, neural, and behavioral (e.g., thirst) responses. The focus on ANG II-dependent mechanisms in the treatment of various cardiovascular diseases has been largely targeted to the effects of ANG II on salt and water balance and on its direct vasoactive properties (25, 55, 88, 111). However, the involvement of ANG II and its membrane receptors in the excitability of autonomic neurons makes it a prime target for central intervention in sympathoexcitatory states such as hypertension and CHF. Finally, interventions such as exercise training (ExT) may also impact sympathoexcitatory outflow in CHF as well as improve buffer reflexes such as the arterial baroreflex.

What is not as well appreciated is the important role that regulation of angiotensin type 1 receptors (AT1Rs) plays in sympathoexcitation in the CHF state. Because the AT1R is thought to be the primary receptor involved in most of the biological effects of ANG II, the degree and mechanism by which it is expressed may provide important insight into the...
sympathoexcitatory process and the development of novel centrally acting therapeutic agents. The bulk of this review will highlight the role of ANG II and downstream signaling molecules in the regulation of neural AT₁Rs and sympathetic nervous function in CHF.

AT₁Rs are found throughout the central nervous system (CNS) and are expressed to a high degree in areas of the hypothalamus and medulla, areas that regulate sympathetic outflow and thus cardiovascular function (2). The AT₁R belongs to a class of G protein-coupled receptors (GPCRs) with seven transmembrane-spanning domains (59). Early work using radioligand binding showed that this receptor binds tightly to ANG II (123). The development of nonpeptide antagonists of the AT₁R not only has provided an enormous therapeutic benefit but also has helped elucidate the many effects of signaling through this receptor. The intracellular signaling pathways that are activated by the AT₁R are vast and incompletely understood. In this review, we describe recent insights in the transcriptional regulation of the AT₁R in the brain of animals with CHF.

**Experimental Outflow in Heart Failure**

Experimental models of CHF, together with clinical data, have solidly established that sympathoexcitation occurs in this disease state and that its genesis resides at various levels in the neuraxis. Clearly, both an elevation in central sympathetic outflow as well as alterations in peripheral norepinephrine (NE) kinetics and metabolism are responsible for the elevated levels of circulating and tissue concentrations of NE (38, 41, 45). Abnormal cardiovascular reflex function contributes to the maintenance of sympathoexcitation in CHF as evidenced by a palliative effect of chronic electrical stimulation of the carotid sinuses in dogs with CHF (165). There is still a general perception that the primary mechanism by which sympathoexcitation occurs in CHF is the reduced sensitivity of various sympathoinhibitory reflexes (e.g., arterial baroreflex and cardiopulmonary reflexes) (57, 74, 94). Studies from this laboratory (12) have shown that arterial baroreflex denervation does not alter the course of the sympathoexcitation or the level of NE in dogs with pacing-induced CHF. Furthermore, Levett et al. (77) demonstrated an increase in plasma catecholamines in dogs with pacing-induced CHF following total cardiac denervation. On the other hand, there has been an underappreciation of the role played by various sympathoexcitatory reflexes in the CHF state. For instance, we (136, 137) and others (24) have shown that an enhancement in peripheral chemoreflex sensitivity (even with normal blood levels of oxygen and carbon dioxide) contributes to both sympathoexcitation and baroreflex dysfunction in CHF. In addition, sympathoexcitatory reflexes originating from visceral and skeletal muscle afferents only recently have been appreciated to contribute to sympathetic activation in CHF (92, 153–155). These studies have demonstrated a significant contribution of the cardiac sympathetic afferent reflex and now skeletal muscle reflexes to augment sympathetic outflow in CHF. While these peripheral cardiovascular reflexes are indeed important contributors to drive sympathetic outflow in CHF, changes in the sensitivity of central sympathetic neurons also occur, which are mediated by alterations in the cellular and molecular environment, including AT₁Rs, and ultimately contribute to the membrane properties of these cells.

**Contribution of Humoral Substances to Sympathoexcitation in Heart Failure**

Overriding the various abnormalities in neural reflexes that are thought to play a role in the sympathoexcitation in the CHF state, circulating and local hormonal factors are capable of modulating sympathetic outflow at several sites in the CNS. Virtually every humoral system or modulator that has been shown to have a sympathoexcitatory or -inhibitory effect is either activated or inhibited in animals and humans with CHF. These include, but are not limited to, ANG II, nitric oxide (NO), reactive oxygen species (ROS), arginine vasopressin, endothelin-1, atrial natriuretic peptide, prostaglandins, and aldosterone. In addition, more recent data show direct central effects of proinflammatory cytokines on sympathetic nerve activity (43, 49, 50, 61, 133, 162).

Blockade of central AT₁Rs reduces sympathetic tone and increases baroreflex function in CHF (31, 32, 102, 166). Studies from our laboratory (52, 53, 84, 85) have shown increases in AT₁R protein, mRNA, and ANG II binding in such sites as the rostral ventrolateral medulla (RVLM) and the nucleus tractus solitarii (NTS) in rabbits and rats with CHF. Figure 1 illustrates differences in AT₁R protein and mRNA expression from punches of the RVLM in sham-CHF and CHF rabbits, demonstrating this point.

Clinical studies also implicate ANG II as an important sympathoexcitatory substance in the CHF state (48, 124, 139). The inability to directly provide evidence for a central component in ANG II signaling in clinical studies is problematic. Techniques such as those described by Aggarwal et al. (1), who evaluated NE turnover in the brain of humans with CHF, come close to probing the central origin of sympathoexcitation in humans; however, these techniques are still limited. Animal studies provide important new evidence for the role of ANG II, AT₁R, AT₂R, selective transcription factors, ACE, ACE2, and ROS on specific central structures in experimental models of CHF (53, 54, 76, 85, 15, 166).

Since the early work on free radical chemistry, there has been an explosion of research related to ROS and their role in the etiology and exacerbation of various diseases at almost every functional and organ system level (35). The role of superoxide anion (O₂⁻) in the exacerbation of many cardiovascular (16, 124) and neurodegenerative (135) diseases is well founded. There is little question that patients and animals with CHF are under oxidant stress (9, 30, 82, 130). For instance, endothelial dysfunction in CHF is, in part, due to a reduction in NO bioavailability because of an increase in O₂⁻ production and conversion of NO to peroxynitrite (7, 39). The role of ROS in neurohumoral control of cardiovascular function is less well understood. However, there is support for the idea of free radical mechanisms playing a role in the regulation of autonomic and baroreflex function in CHF. In an important study, Li et al. (81) revealed that oxygen-derived free radicals impaired baroreceptor function. A recent study by Piccirillo et al. (113) confirmed that chronic antioxidant therapy with vitamin C in patients with CHF enhanced baroreflex function. Additional evidence that implicates ROS generation in cardiovascular reflex function comes from the laboratory of Schultz and...
coworkers (147–150). In these studies it was clearly shown that oxygen-derived free radicals contribute to abnormal afferent and reflex function in normal and diabetic animals during the late reperfusion phase following a period of cardiac ischemia. Finally, data are accumulating to suggest that important integrative pathways in the medulla, hypothalamus, and forebrain of animals with CHF and hypertension are under significant oxidant stress (75, 82, 83). For instance, Zanzinger and Czachurski (160) have carried out experiments in anesthetized swine strongly suggesting that oxidative stress activates sympathetic neurons in the RVLM.

It is well established that ANG II is a potent stimulator of ROS (58). ANG II has more recently been implicated in activation of ROS in CHF (85, 124). The mechanism by which ANG II stimulates $O_2^{•−}$ production is related to activation of NAD(P)H oxidase in most tissues in which it has been examined (57). For instance, Oudot et al. (108) demonstrated activation of cardiac NAD(P)H oxidase in isolated, perfused hearts in response to ANG II. In a recent study by Privratsky et al. (118) conducted in isolated myocytes incubated with high glucose, protection from myocyte contractile dysfunction occurred after AT$_1$R blockade. This effect could be mimicked by the NAD(P)H oxidase inhibitor diphenyleneiodonium (10 μmol/l) or apocynin (100 μmol/l). Furthermore, high glucose induced an upregulation of p47$^{phox}$, one of the protein subunits of NAD(P)H oxidase. This upregulation could be blocked by AT$_1$R blockade. We have provided evidence (51, 52, 84) that similar mechanisms function in the RVLM to activate sympathetic tone in normal animals infused with intracerebroventricular ANG II and in CHF rabbits. Figure 2 shows upregulation of several of the protein subunits of NAD(P)H oxidase in the RVLM of rabbits after chronic intracerebroventricular infusion of ANG II (53). Interestingly, this upregulation, as well as that of the AT$_1$R, could be completely reversed after intracerebroventricular treatment with the AT$_1$R antagonist losartan (53, 84). The increase in NAD(P)H oxidase protein subunits in rabbits with CHF has also been demonstrated (Fig. 3), and the production of NAD(P)H oxidase-dependent $O_2^{•−}$ in the RVLM could be inhibited by administration of superoxide dismutase (SOD) (51). Furthermore, intracerebroventricular administration of losartan reduces NAD(P)H oxidase superoxide production in response to ANG II (53). These data strongly suggest that upregulation of the AT$_1$R in the RVLM is dependent on ANG II binding and activation of downstream pathways for its expression and synthesis.

**Transcriptional Regulation of Neuronal AT$_1$R Expression**

The regulation of AT$_1$R expression in peripheral tissues has been investigated for some time (71, 75) but has not been clearly defined in the CNS, especially in sympathoexcitatory pathways.
disease states such as hypertension and CHF. Chan et al. (19, 20) demonstrated an important contribution of c-fos to AT1R upregulation in the NTS and RVLM of normal and spontaneously hypertensive rats (SHR). This same group showed involvement of the p38 MAPK and ERK1/2 pathways in the RVLM (20, 21). Chan et al. (21) also implicated O2•− and the NAD(P)H oxidase pathway as mediators of AT1R signaling and regulation. These data are, in part, consistent with our observations (51, 84, 85) in animals with CHF in which upregulation of the transcription factor activator protein 1 (AP-1; c-jun) along with the AT1R was observed in the RVLM of rabbits with CHF. Figure 4 shows an increase in AP-1-DNA binding in the RVLM of rabbits with CHF, suggesting that activated AP-1 translocates to the nucleus in animals with CHF. Other transcription factors such as NF-κB, Creb, and Raf-1 also have been associated with activation of the AT1R gene (67, 69, 91). Although these entities have been investigated in a variety of cell types in hypertension, surprisingly there has been little work conducted in neurons that regulate sympathetic outflow in CHF. As indicated above, it is clear that ANG II-induced upregulation of AT1R gene expression depends on ANG II binding to the AT1R (36, 53, 84, 85) since its upregulation is blocked by losartan. It is important to point out that this signaling pathway constitutes a positive feedback mechanism that could contribute to the pathological consequences of AT1R upregulation in CHF.

There is ample evidence to support the idea that, at some level, the transcriptional regulation of some proteins is redox sensitive (9, 10, 72, 164). Because there is oxidative stress in the periphery and in the CNS in the CHF state, the question of redox-sensitive transcriptional regulation of AT1R expression is relevant. In a study designed to address this issue Liu et al. (85) chronically infused the antioxidant Tempol directly into the CNS (icv) of rabbits with pacing-induced CHF. Intracerebroventricular Tempol infusion reduced AT1R expression and decreased the activity of AP-1 and its binding to DNA in tissue from the RVLM of rabbits with CHF (Fig. 5). These data along with the demonstration that intracerebroventricular losartan decreases both oxidative stress and the expression of several subunits of NADPH oxidase (53) suggest that an increase in central oxidative stress generated in CHF by ANG II-NADPH oxidase may participate in the regulation of AT1R expression by modulating transcription factor production and binding, thus contributing to the positive feedback nature of central sympathoexcitation. The importance of oxidative stress in AT1R expression is further demonstrated by studies showing a loss of both manganese (Mn)- and copper/zinc (CuZn)-SOD in various tissues in CHF. Gao et al. (54) clearly showed a decrease in both enzymes in the RVLM of rabbits with pacing-
induced CHF. Furthermore, adenoviral upregulation of CuZn-SOD in the RVLM reduced resting renal sympathetic nerve activity (RSNA) in CHF rabbits but had little effect in sham-CHF animals.

ACE2, ANG-(1–7), and Sympathoexcitation

The carboxypeptidase ACE2 was originally discovered in yeast (17) as a gene product that codes for a protein that is a homolog of the more widely known protease ACE. A mammalian homolog of the yeast enzyme was then discovered by Tipnis et al. (143) and by Douglas et al. (34). ACE2 cleaves phenylalanine from the carboxy terminal end of the octapeptide ANG II to form the heptapeptide ANG-(1–7). The actions of ANG-(1–7) are diverse and have been examined in many tissues. In the heart, ANG-(1–7) is expressed in cardiac myocytes, appears to have an ionotropic effect, and possesses coronary vasodilator activity (3, 14, 40). The peptide has been shown to possess vasodilator activity (14) and stimulates the Mas oncoreceptor (140). In contrast to other tissues both ACE and ACE2 appear to be elevated in the heart of animals with CHF (15, 37). In the CNS the role of ACE2 and ANG-(1–7) is not as straightforward. The action of ACE2 and ANG-(1–7) has been the subject of several recent reviews (22, 112, 116, 146). ANG-(1–7) is widely produced in the brain (23), but its actions in the brain relate primarily to sympathetic regulation and vasopressin release (119, 125, 126). The effect of ANG-(1–7) on sympathetic outflow is controversial and not well understood. Potts et al. (117) showed that in anesthetized rabbits ANG-(1–7) exhibited a sympathoexcitatory action when administered into the RVLM, although at much higher doses than that of ANG II. Similar results were obtained by Fontes et al. (47) and by Silva et al. (129) in the rat. In a more recent study, Da Silva et al. (27) injected the Mas receptor antagonist A-779 in the paraventricular nucleus of anesthetized rats and observed a decrease in RSNA, suggesting that ANG-(1–7) is transiently sympathoexcitatory compared with ANG II. On the other hand, several studies suggest that ANG-(1–7) may be sympathoinhibitory. Studies by Ferrario et al. (46) suggest a sympathoinhibitory effect of ANG-(1–7). Gironacci et al. (56) showed that ANG-(1–7) decreased NE release from the hypothalamus of SHR. In a recent study by Yamazato et al. (159), overexpression of ACE2 by lentiviral transfection of the RVLM reduced arterial pressure only in SHR. Recent evidence suggests that overexpression of ACE2 initiates a decrease in AT1R expression in a neuronal cell line (Neuro-2A) (44). In a recent study by Campagnole-Santos and Guimaraes (18), intracerebroventricular infusion of ANG-(1–7) reduced blood pressure and heart rate in DOCA-salt hypertensive rats. Therefore, these data suggest a novel regulatory pathway for ANG-(1–7). They suggest the potential for the balance between ACE and ACE2 in the brain to influence sympathetic function. These data also indicate a potentially important counterregulatory mechanism of AT1R expression in neurons. The regulation of angiotensin receptor expression by ANG-(1–7) clearly warrants further investigation.

Exercise Training and Heart Failure

Over the past several years, ExT has become an important therapeutic modality capable of enhancing the quality of life of patients with CHF (28, 29, 95, 97, 99). More recent data have shown that a long-term ExT program prolongs life and reduces adverse effects including hospitalization in patients with CHF (8, 114). The mechanism(s) by which ExT is beneficial to patients with CHF are not well understood. While ExT has been shown to enhance endothelial function in patients and animals with CHF (68, 99, 127, 144, 152, 157), there is a
paucity of data on the role of ExT in the modulation of neurohumoral function in CHF. In a recent review on this issue the focus was primarily on enhanced skeletal muscle perfusion and morphology as a mechanism for improvement (42). The current ongoing HF-ACTION (156) trial will enroll over 3,000 CHF patients to evaluate the safety and efficacy of ExT. This clinical trial should shed light on potential beneficial mechanisms of ExT in CHF.

In both humans and animals in the normal state, ExT increases cardiac vagal tone and reduces sympathetic outflow at rest (26, 33, 73). On the other hand, arterial baroreflex sensitivity has been reported to be reduced (93, 131), increased (132), or unchanged (116, 128) in subjects following a course of ExT. It is still a bit unclear as to which autonomic components are altered after ExT, especially in the setting of CHF in humans.

Much of our understanding of brain mechanisms regulating sympathetic tone after ExT in CHF comes from work carried out in animal models in our laboratory and in humans in the studies of Negrao, Middlekauff, and colleagues (103, 104, 122). We have shown (54, 78, 86) that ExT reduces RSNA in conscious animals with experimental CHF. Both reflex and central mechanisms contributing to elevated sympathetic outflow in CHF are abrogated by ExT (54, 79). The central mechanisms responsible for decreasing sympathetic outflow after ExT in CHF are, in part, dependent on changes in ANG II, AT1Rs, and ROS (54, 86, 101). For instance, in rabbits with pacing-induced CHF, ExT results in a decrease in plasma ANG II (86, 101), a decrease in AT1Rs, and ROS (54, 86, 101), increased (132), or unchanged (116, 128) in subjects following a course of ExT. It is still a bit unclear as to which autonomic components are altered after ExT, especially in the setting of CHF in humans.

Unanswered Questions

The information summarized above is a snapshot of what is currently known concerning the transcriptional regulation of AT1R in the RVLM and the role of at least one important intervention, ExT, on both sympathoexcitation and central AT1R mechanisms in the CHF state. Admittedly, many intriguing questions remain to be answered before a complete picture of this system can be drawn. First, the specific cellular elements in the RVLM (and other nuclei) that participate in the regulation of the AT1R expression in CHF need to be defined. Similarly, the cellular distribution of ACE and ACE2 has not been resolved. Second, the roles of AT1R-Rs in both the regulation of AT1R expression and the antagonism of AT1R signaling in the setting of CHF are important areas of future investigation. Finally, it is still unclear exactly how ExT influences

![Diagram](http://ajpheart.physiology.org/)

Fig. 6. Schematic overview of some of the neuronal angiotensin-dependent signaling pathways that may participate in the sympathoexcitatory process in the setting of CHF. Some of these targets can be modulated by exercise training, which may reduce sympathetic outflow in CHF. O2•−, superoxide anion; SOD, superoxide dismutase; H2O2, hydrogen peroxide; JNK, jun NH2-terminal kinase; ACE, angiotensin-converting enzyme. Solid line, excitatory; dashed line, inhibitory.
sympathetic tone, AT1R function, and ROS in the brain of animals with CHF.

Summary and Conclusions

The regulation of sympathetic nerve activity is complex even in normal conditions. In the setting of CHF this regulation is complicated by multiple neurohumoral abnormalities. The data summarized above strongly indicate that central AT1R upregulation plays a critical role in setting the sensitivity of presynaptic neurons residing in the RVLM and, most likely, in other areas involved in central sympathetic control. The enhancement of neuronal AT1R transcription appears to be a critical mechanism of this central AT1R upregulation. As summarized in Fig. 6, several factors including oxidative stress impact on AT1R expression and activity, which, in turn, modulate neuronal activity. Targeting AT1R modulation and its downstream regulatory molecules in the brain may be a worthwhile therapeutic strategy in CHF, hypertension, and other hyperadrenergic states.

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