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# The Central RAS and Sympathetic Nerve Activity in Chronic Heart Failure

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## Abstract

Chronic heart failure (CHF) is a multi-factorial disease process that is characterized by over activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system. Both of these systems are chronically activated in CHF. The RAAS consists of an excitatory arm involving Angiotensin II (Ang II), Angiotensin Converting Enzyme (ACE), and the Ang II type 1 Receptor (AT1R). The RAAS also consists of a protective arm consisting of Angiotensin-1-7 (Ang -1-7), the Ang II type 2 receptor (AT2R), ACE2 and the mas receptor. Sympathoexcitation in CHF is driven, in large part, by an imbalance of these two arms, with an increase in the Ang II-AT1R-ACE arm and a decrease in the AT2R-ACE2 arm. This imbalance is manifested in cardiovascular-control regions of the brain such as the rostral ventrolateral medulla and paraventricular nucleus in the hypothalamus. This review focuses on current literature that describes the components of these two arms of the RAAS, and their imbalance in the CHF state. Moreover, this review provides additional evidence for the relevance of ACE2 and Ang-1-7 as key players in the regulation of central sympathetic outflow in CHF. Finally we also examine the effects of exercise training as a therapeutic strategy and the molecular mechanisms at play in CHF, in part, because of the ability of exercise training to restore the balance of the RAAS axis and sympathetic outflow.

## Introduction

Chronic heart failure (CHF) is the end result of various insults to the myocardium, the most common of which is ischemic heart disease [1]. Whenever cardiac output falls, more than momentarily, compensatory mechanisms are called into play in an attempt to maintain blood pressure and organ perfusion. Primary to this compensation is activation of the sympathetic nervous system and the renin angiotensin aldosterone system (RAAS). Increases in circulating norepinephrine (NE) and Angiotensin II (Ang II) evoke peripheral vasoconstriction and activate aldosterone secretion largely through α-adrenergic and Ang II Type 1 (AT1) receptors, respectively. An increase in renal renin release is undoubtedly caused by renal sympatho-excitation as well as a decrease in renal perfusion pressurein the CHF state[2-4]. While these compensatory changes are initially beneficial they become counterproductive if sustained for prolonged periods of time. Chronic increases in both

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plasma and tissue NE not only result in a down-regulation of β-adrenergic receptors in the heart[5, 6] but also contribute to further myocyte death[7]. Activation of the RAAS contributes in a major way to salt and water retention and to further sympatho-excitation[8, 9].

## Central AT1R in Heart Failure

A large body of evidence points to the central RAAS in mediating sympatho-excitation in the setting of CHF[3, 10-14]. Areas in the rostral ventrolateral medulla (RVLM) and in the hypothalamus, (e.g. the paraventricular nucleus (PVN)) have been shown to mediate an increase in sympathetic outflow in response to microinjection of Ang II[15-25]. In studies carried out in rabbits with pacing-induced CHF Liu et al.[26] showed that central infusion of the AT1R blocker losartan reduced renal sympathetic nerve activity (RSNA). Furthermore, Zucker and co-workers showed an increase in Ang II in the cerebral spinal fluid of dogs with pacing-induced CHF[27] compared to sham animals. In an attempt to further understand the role of central AT1R in the sympathoexcitatory process, Gao et al. [28]measured AT1R protein and message in the RVLM of rabbits with pacing-induced CHF, both of which were increased (figure 1). Interestingly, in this and other studies[29] it was shown that this increase was associated with increased oxidative stress and RSNA in these animals. Similar observations have been made in rats with coronary artery ligation-induced CHF[30, 31]. In the rat model, Zhu et al.[32] carried out a novel study in which rats were infused with the antisense oligonucleotide to the AT1R while recording RSNA and blood pressure (figure 2). Compared to animals infused with scrambled antisense inhibition of the AT1R resulted in a decrease in RSNA. Upregulation of central AT1R is not limited to the RVLM and PVN in CHF; the subfornical organ (SFO), nucleus of the solitary tract (NTS), and area postrema (AP) are other areas of the brain have also shown an upregulation of AT1R in the setting of CHF[33, 34].

The ability of the central RAAS to be a key player in the reverberating circuit of heart failure is not limited to the central nervous system. Indeed, intracerebroventricular (icv) blockade of the angiotensin receptor improved baroreflex sensitivity and decreased efferent renal sympathetic nerve activity in CHF rats[35, 36]. Central blockade of angiotensin converting enzyme (ACE) similarly decreased renal sympathetic nerve activity, improved the blunted baroreflex sensitivity, and normalized sodium consumption, urine sodium and urine volume in CHF rats[11]. Further, in a myocardial infarction rat model with a transgenic deletion of angiotensinogen, left ventricular enddiastolic pressure did not increase to the same extent as control CHF rats and LV dp/dt max did not decrease to the same extent as control CHF rats[37]. Taken together, the hyperactive central RAAS is a contributor to the global physiological changes as well as the cardiovascular dysfunction seen in CHF.

This apparent increase in AT1R signaling in the CNS appears to be mediated by a positive feedback of AT1R on the transcriptional regulation of the protein. The AT1R is upregulated in response to Ang II and in the CHF state[17, 29, 38, 39]. In a neuronal cell line (CATH.a) that express AT1Rs Mitra et al.[40] showed that in response to Ang II (100 nM), an NFkB-dependent increase in AT1R transcription ensued. This increase was also dependent on the downstream activation of two additional transcription factors, namely Ets-like kinase 1

(Elk-1) and activator protein 1 (AP-1). This pathway was confirmed in intact rabbits with CHF in which c-jun (one of the two transcription factors that dimerizes to form AP-1) and Jun N-terminal kinase (JNK) was increased in the RVLM[38]. The NFkB pathway has been shown to mediate an increase in sympathetic nerve activity in CHF since its blockade reduces sympathetic outflow, AT1R expression and oxidative stress in rats with CHF and hypertension[30, 41, 42]. This sympatho-excitation in response to central Ang II is most likely mediated by a decrease in the outward potassium current[43-45]. Recently, Gao et al. [46] showed that a potassium channel protein, Kv4.3 was downregulated in the brainstem of rats with CHF suggesting that this may contribute to enhanced membrane depolarization and action potential generation. Kv4.3 contributes to the transient outward current and is most prominently reduced in cardiac myocytes in CHF[47, 48]. The mechanism by which Ang II decreased $K^+$ current is not clear but may result from interaction of a Kv4.3-AT1R complex[49].

## A Role for Angiotensin Converting Enzyme (ACE) 2 in the Sympatho-Excitatory Process

The discovery of ACE2 and generation of Ang 1-7[50, 51] as important components of the RAAS has resulted in an explosion of studies on the biological and therapeutic effects of this arm of the RAAS. ACE2 activation has been shown to have beneficial effects in a variety of disease states[52]. Furthermore, Ang 1-7 has been shown to be beneficial in the setting of systemic hypertension[53, 54], pulmonary hypertension[55], renal disease[56] and cancer[57-59]. Since ACE2 has been found in the brain[60, 61] it seems reasonable that this enzyme and its product, Ang 1-7, would modulate the generation of central sympathetic outflow in CHF. Utilizing a unique transgenic mouse model that overexpresses human ACE2 selectively in neurons[53]Xiao et al.[62] examined the sympathetic nervous effects in transgenic and wild type mice subjected to a chronic myocardial infarction and the subsequent development of CHF. While there were no major differences in cardiac function in both groups of mice, transgenic mice exhibited a lower RSNA and an improvement in arterial baroreflex function (figure 3). Mice that overexpressed central ACE2 were able to suppress RSNA to zero during increases in blood pressure in contrast to wild type mice with CHF who could not lower RSNA in response to an increase in blood pressure. Examination of the spontaneous baroreflex control of heart rate also indicated an enhanced sympatho-inhibitory process in these mice. In a recent study by Zheng et al.[63] it was shown that viral overexpression of ACE2 reduced RSNA in a rat CHF model. This effect was apparently mediated by an increase in nitric oxide.

In an attempt to understand the cellular mechanisms by which over expression of ACE2 in neurons regulates AT1R expression we carried out *in vitro* studies where the neuronal cell line, CATH.a was transfected with a lentivirus that resulted in overexpression of human ACE2. As can be seen in figure 4, Ang II stimulated an increase in AT1R expression which was blocked by the AT1R antagonist, losartan. Overexpression of ACE2 completely prevented the increase in AT1R in response to Ang II. Interestingly, this suppression was not reversed by the mas receptor antagonist A-779. These results suggest that the efficacy of ACE2 to prevent the upregulation of AT1R may be related to its ability to degrade Ang II rather than to the generation of Ang 1-7. Because over expression of human ACE2 may be quite nonphysiologic and expression may be increased by several fold over endogenous

mouse ACE2, the effects of exogenous Ang 1-7 was tested in order to determine if this peptide could alter AT1R expression *in vitro*. Figure 5 shows the results from this experiment. Again losartan prevented the upregulation of the AT1R in response to Ang II. Ang 1-7 also increased AT1R expression. Both the response to Ang II and the response to Ang 1-7 were blocked by losartan and neither was blocked by A-779. Taken together these data strongly suggest, at least *in vitro*, that Ang 1-7 does not prevent the up regulation of the AT1R and that the efficacy of ACE2 in this response is mediated by an AT1R dependent reduction in Ang II. Therefore, increases in Ang 1-7 may be secondary to a reduction in AT1R activation as a therapy in the setting of CHF. However, therapies that both stimulate the Ang 1-7 pathway and decrease AT1R would be of additive benefit. It is also possible that Ang 1-7 signaling through the mas receptor augments nitric oxide production which has been shown to decrease AT1R expression[64] .

While manipulations of ACE2 may provide insight into the autonomic effects of this enzyme they do not directly address the question of whether Ang 1-7 is capable of modulating sympathetic nerve activity in a beneficial direction, especially in cardiovascular disease states. There has been controversy over the effects of Ang 1-7 on sympathetic outflow and baroreflex function. In early experiments Potts et al.[65] and Da Silva et al.[66] provided evidence for a sympatho-excitatory effect of Ang 1-7. On the other hand, Xia et al. [67] and Diz et al.[68] have provided evidence that Ang 1-7 exerts sympatho-inhibitory effects. In a recent study by Kar et al.[69] conscious rabbits with and without CHF were infused by the intracerebroventricular (icv) route with Ang 1-7 for several days. Autonomic balance to the heart was assessed by evaluating the heart rate response to an acute bolus injection of either atropine to assess vagal tone or metoprolol to evaluate cardiac sympathetic outflow. Ang 1-7 increased vagal tone in CHF rabbits (i.e. a greater increase in heart rate in response to atropine) and decreased sympathetic tone (a smaller decrease in heart rate in response to metoprolol). There was no effect of Ang 1-7 in sham rabbits. In addition, chronic icv infusion of Ang 1-7 profoundly reduced RSNA in conscious CHF rabbits while having no effect in normal rabbits. Importantly, this effect was mediated by the mas receptor since it was reversed when Ang 1-7 was co-infused with A-779 (figure 6). Consistent with these results baroreflex gain for both heart rate and RSNA was increased in rabbits with CHF.

## Modulation of Central RAS Components by Exercise Training in CHF

There is a growing trend to consider the use of non-pharmacological therapy in the treatment of CHF. One such intervention is exercise training (ExT). In 2003 a position statement by The American Heart Association concluded that ExT of patients with CHF is safe and likely to be an effective treatment paradigm[70].There is now clear evidence that ExT of patients with CHF increases quality of life and improves survival from all cardiac events[71-74] even if cardiac function *per se* is not improved. However, in elderly patients the benefits may not be as great[75, 76]. In experiments carried out in rabbits with pacing-induced CHF, Mousa et al.[77] and Liu et al.[78] showed that ExT reduced AT1R expression in the RVLM and reduced plasma Ang II. Similar results have been reported in rats with MI-induced CHF[79]. In humans, studies by Roveda et al.[80] and by Negrão and colleagues[81-83] have clearly shown a decrease in muscle sympathetic nerve activity (MSNA) in CHF

patients following ExT. These studies raise important questions concerning the role of the central RAAS in mediating the sympatho-inhibitory effects of ExT in the setting of CHF. What does ExT do to central Ang II signaling and to the components of the RAAS system in the central nervous system? Exercise training in experimental CHF has been shown to reduce central AT1R[77, 84] and oxidative stress[85-87]while at the same time increasing the sympatho-inhibitory effects of nitric oxide[84, 88] and improving baroreflex function[77, 78, 86, 89]. There is much less known about ACE and ACE2 in the central nervous system following ExT in the CHF state. In a study carried out in rabbits with pacing-induced CHF Kar et al.[90] examined the relationship between ExT and the expression of ACE and ACE2 in the RVLM and PVN. In both regions ACE was elevated in the CHF state and ACE2 was decreased. Following ExT the expression of these two proteins were reversed and looked very similar to sham animals. While there are several studies showing that ExT reduces ACE in the myocardium[91, 92] the above is the first study showing a reversal of the ACE/ACE2 ratio in the brain of animals with CHF. Because Ang 1-7 is thought to be sympatho-inhibitory and Ang II sympatho-excitatory, a decrease in the ratio of ACE to ACE2 should be beneficial in reducing sympathetic outflow in CHF and hypertension. This in turn would also reduce oxidative stress and increase nitric oxide at the local level. Theoretically, the ACE/ACE2 balance would also mediate the relative concentrations of Ang II and Ang 1-7 in the brain. *In vitro* evidence has shown that Ang 1-7 can mediate an increase in neuronal potassium current by a nitric oxide and mas receptor-dependent mechanism[93].

The expression of AT1Rs in the brain is critically tied to signaling through the AT1R in animals with CHF. This positive feedback nature was shown in CHF rabbits that underwent an ExT regimen while simultaneously receiving a chronic infusion of Ang II so that plasma Ang II would not be normalized[77]. Under these conditions AT1R message and protein in the RVLM remained elevated (compared to non-ExT CHF rabbits). Furthermore, resting RSNA and arterial baroreflex function remained elevated and depressed, respectively. These data, along with those from chronic central losartan infusion, strongly suggest that both high levels of Ang II and increased AT1R signaling are necessary for sympatho-excitation and that the normalization of these parameters by ExT are mediated, at least in part, by a reduction in both.

## Regulation of Central RAS Components

Because plasma Ang II is reduced following ExT in CHF animals[39] (although it is unclear if this is also true for Ang II in the brain) it is of some interest to determine the influence of Ang II on ACE and ACE2 expression. In a recent *in vitro* study, Xiao et al.[94] clearly showed that Ang II treatment of neurons resulted in an increase in ACE and a decrease in ACE2 in a dose-dependent manner. At the message level the changes for both proteins in response to Ang II could be inhibited by blocking p38 MAPK or ERK1/2. This seems to be selectively mediated by the AT1R as it was also blocked by losartan but not by the AT2R blocker PD123319. This reciprocal relationship appears to hold in other tissues and in other diseases states such as hypertension[95- 98]. These data suggest that Ang II can set into motion a series of transcriptional events through common cell signaling pathways to

regulate the balance between ACE and ACE2. However, the transcription factors that drive the regulation of both ACE and ACE2 have not yet been identified.

While the transcription of new AT1R protein may be an important contributor to central Ang II signaling and another mechanism of potential importance relates to the way the AT1R is turned over. The AT1R is a G-protein coupled receptor and signals through a Gq/11 and other G protein mechanisms[99]. As such, its phosphorylation following agonist binding is mediated by G-protein receptor kinases (GRK)[100]. Following phosphorylation the protein is targeted for internalization by β-arrestin after which it is degraded in lysosomes[101]. Recent experiments carried out in rats with CHF have shown that GRK5 is upregulated in the RVLM and PVN (figure 6) and binds to the AT1R[79]. On the other hand, GRK2, the more classical β-adrenergic receptor kinase that can also regulate AT1R is not changed. Interestingly, the increase in GRK5 occurs at the same time as the AT1R is upregulated. Following ExT both GRK5 and AT1R were decreased. This parallel change in both proteins suggests that the increase in GRK5 is a compensatory response to the increased AT1R expression. The increase in GRK5 may not be effective in decreasing AT1R expression due to intense stimuli that increase the transcription of this receptor (e.g. NFkB) in CHF.

*In vitro* experiments carried out in CATH.a neurons confirmed that substantial upregulation of GRK5 results in a decrease in AT1R protein. Under these conditions, the Ang II – mediated increase in AT1R expression was completely blocked (Figure 7). On the other hand, GRK5 knock down with an siRNA caused an increase in AT1R expression in response to Ang II. Taken together these studies suggest that a balance exists between the transcriptional regulators of AT1R and the pathways responsible for degrading the AT1R. In the setting of CHF the former apparently predominate, thus promoting an Ang II-dependent neuronal depolarization and increase in sympathetic outflow.

Despite our increasing knowledge on the regulatory pathways of AT1R and central RAS components, the induction of the central RAS, especially in regions with an intact blood-brain barrier like the RVLM, remain unclear. While angiotensinogen is found in brain extracellular fluid and cerebrospinal fluid, astrocytes and more recently in the neurons of many brain regions including the PVN, NTS, RVLM and SFO, the cell types in which renin, ACE, aminopeptidase A and aminopeptidase N are found in the brain are still controversial. To date only low levels of Ang I, Ang II and Ang 1-7 have been identified in brain tissue[22]. Therefore, one possibility is that the Ang II from the periphery detected in circumventricular organs (CVOs) would induce a signaling cascade in non-CVO nuclei. Conversely, given that much of the RAS is expressed centrally between neurons, astrocytes and endothelial cells, angiotensins are not only present in the brain, but may function as neurotransmitters[102] .

Data from this laboratory and others strongly suggests that circulating Ang II is a primary driver of the imbalance for AT1R-ACE and AT2R-ACE2. Both *in vitro* and *in vivo* studies have demonstrated that Ang II mediated increase in AT1R and its pathway components are dependent on AT1R signaling; pretreatment with Losartan blunts this imbalance[29, 38].

## Summary

Clearly, the regulation of sympathetic nerve activity is a complicated and multifactorial process. This review only highlights one mechanism that plays a role in this process. Ang II along with other peptide and non-peptide mediators can alter neuronal membrane potential, in part, by reducing outward potassium currents. Most central pre-sympathetic neurons express all the receptors involved in signaling through the RAS. Therefore, the balance between Ang II and other peptides such as Ang 1-7 and the balance between AT1R, AT2R and mas receptors may be critical to establishing the level of neuronal activation. Furthermore, the synthesis of Ang II and Ang 1-7 due to ACE and ACE2 also appears to contribute to sympatho-excitation in CHF. Figure 8 outlines the major mechanisms in the RAS system that has thus far been defined to regulate sympatho-excitation in CHF. Increases in central Ang II initiate a positive feedback process whereby the AT1R is upregulated by an Ang II-AT1R-dependent mechanism. Intracellular activation of NFkB and downstream transcription factors (Elk-1 and AP1) increase the transcription of the AT1R. This process is accompanied by a compensatory increase in GRK5 in an attempt to limit AT1R upregulation. However, the apparent intensity of the stimuli to up regulate this protein far outweighs the ability of GRK5 alone to reduce the AT1R protein.

The relative paucity of new pharmacological agents in the treatment of CHF has stimulated a search for non-pharmacological therapies. In addition to novel device therapy (e.g. baroreflex stimulation, renal denervation, vagal stimulation), ExT has been promoted as a way of reducing mortality and increasing the quality of life for patients with CHF. The mechanisms by which ExT is efficacious in this regard are not well understood. While ExT is known to affect virtually every organ system, the focus on central sympathetic remodeling is starting to define some of the pathways impacted by this intervention[103]. ExT impacts the central RAS in a major way. Importantly, it reduces oxidative stress in the RVLM and causes an upregulation of both SOD1 and SOD2[86]. Since Ang II signals, in part, through the NADPH oxidase – dependent production of superoxide, ExT is likely to have a major impact on Ang II signaling. Indeed ExT lowers plasma Ang II and reduces AT1R expression which is dependent on activation of the AT1R (thus, positive feedback). Furthemore, ExT reduces ACE and increases ACE2 in the setting of CHF. Therefore, the role of ExT in modulating the central RAS would seem a fruitful area of continued investigation. This simple and inexpensive intervention may provide some of the benefits of currently used pharmacological therapies and can also be used as adjunctive therapy.

### Future Directions

Many questions still remain in the regulation of central RAS in the setting of CHF. It will be important to determine the precise location and mechanism(s) by which central Ang II is generated, and what initiating signal drives the feed-forward activation of the RAS in the setting of CHF. Conversely, the precise central and peripheral signals generated by ExT that trigger the downstream effects outlined in this review that lead to protection in the setting of CHF also remain unclear. Additionally, the mechanism(s) by which nitric oxide can negatively regulate AT1R are still unknown. Finally, because many existing therapies that

target the RAS do not improve cardiac parameters, it will be important to develop novel therapies that both improve autonomic imbalance and hemodynamic parameters.

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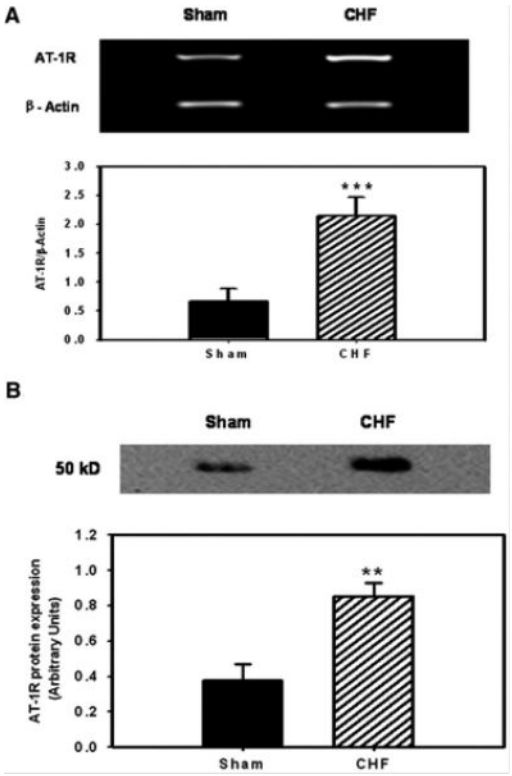



**Figure 1.**

A, RT-PCR analysis for mRNA expression of the AT1 receptor in the RVLM of sham and CHF rabbits. Top, A representative RT-PCR image showing the upregulated AT1 receptor mRNA expression in the RVLM of a CHF rabbit. β-Actin was used as internal control. Bottom, Results of densitometric analysis representing means±SE. ***P<0.001 compared with sham; n=9 each group. B, Western blot analysis for protein expression of the AT1 receptor in the RVLM of sham and CHF rabbits. Top, Representative Western blots showing the upregulation of AT1 receptor protein expression in RVLM of CHF rabbit. Bottom, Results of densitometric analysis representing means±SE. **P<0.0 compared with sham; n=8 each group. From Gao et al[28].

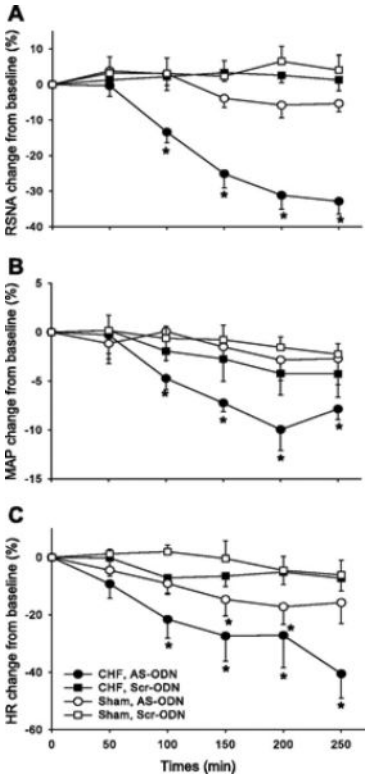



**Figure 2.**

Effects of intracerebroventricular (ICV) administration of antisense oligodeoxynucleotides (AS-ODN) and scrambled oligodeoxynucleotides (Scr-ODN) on baseline renal sympathetic nerve activity (RSNA; *A*), mean arterial pressure (MAP; *B*), and heart rate (HR; *C*) in sham-operated (Sham) and chronic heart failure (CHF) rats. AS-ODN significantly decreased baseline RSNA, MAP, and HR ($n = 7$ for each group) over time. Values are means ± SE. *$P < 0.05$ compared with administration of Scr-ODN. From Zhu et al[32]. Reprinted with Permission from the American Journal of Physiology.

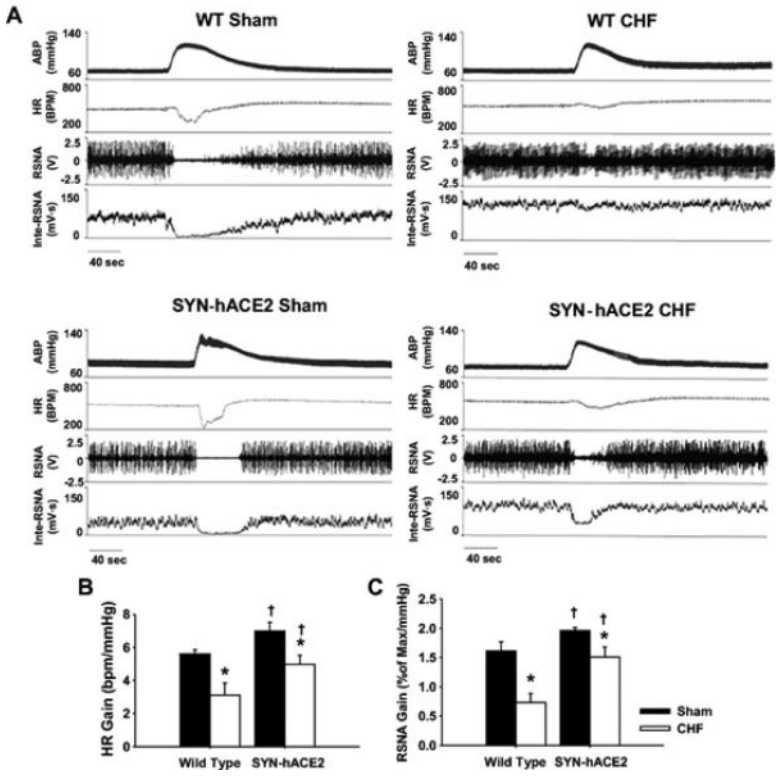
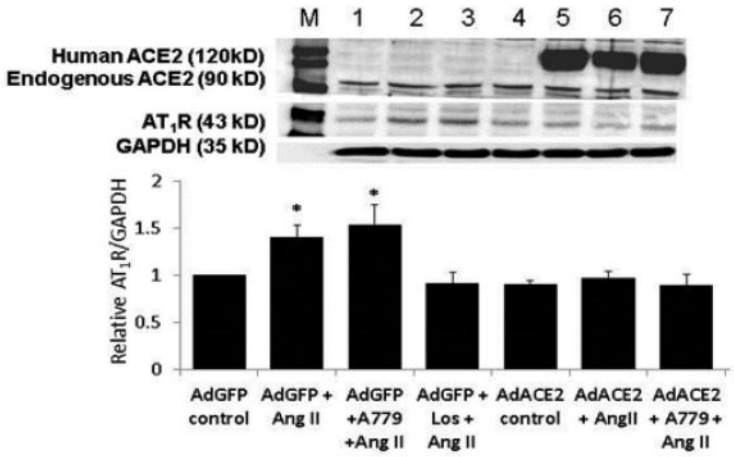



**Figure 3.**

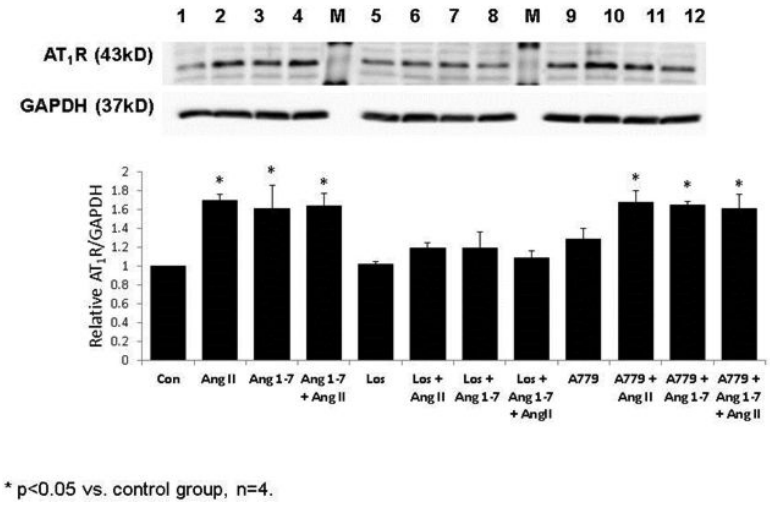
Baroreflex response to elevation in blood pressure induced by phenylephrine. A, Representative recordings for arterial blood pressure (BP), heart rate (HR), raw renal sympathetic nerve activity (RSNA), and integrated RSNA from anesthetized wild-type (WT) and SYN-hACE2 mice with either sham surgery or chronic heart failure (CHF). Mean values of the gain for HR and RSNA in each group are shown in B and C. *P<0.05 vs the corresponding group in sham mice; †P<0.05 vs the WT-CHF group. n=4 to 5 per group. From Xiao et al[62]




* p<0.05 vs. control group, n=4.

**Figure 4.**

AT1R protein expression in Cath.a neurons which were incubated with Ang II alone or in combination with either a mas receptor antagonist, A-779 or an AT1R receptor antagonist, losartan. Experiments were conducted with either a GFP adenovirus or human ACE2 adenovirus. ACE2 overexpression inhibited the upregulation of the AT1R in response to Ang II however this was not reversed by A-779. Data generated by Dr. Liang Xiao.




* p<0.05 vs. control group, n=4.

**Figure 5.**

AT1R expression is upregulated by both Ang II and Ang (1-7) in CATH.a cultured neurons. Losartan but not A-779 blocked the response to both peptides. Data generated by Dr. Liang Xiao.

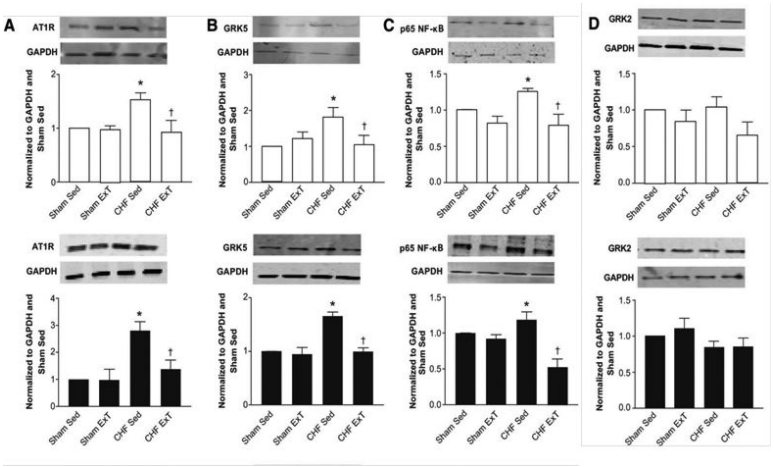



**Figure 6.**

AT1R (A), GRK5 (B), p65 NF-κB (C) are increased in the PVN (solid bars) and RVLM (open bars) of CHF animals and normalized by ExT. GRK2 (D), another kinase implicated in regulating AT1R expression, is unchanged in the PVN during both CHF and ExT. **P*<0.05 versus Sham-Sed. †*P*<0.05 versus CHF-Sed; n=5 to 7. From Haack et al[79].

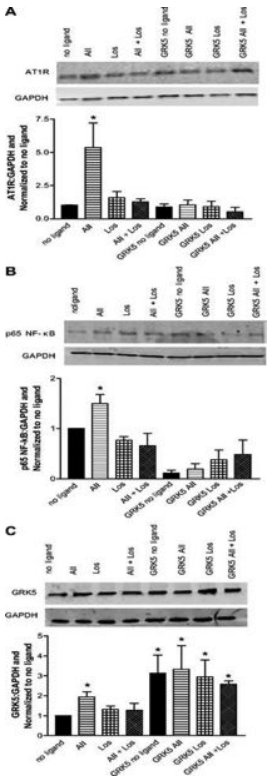



**Figure 7.**

GRK5 overexpression normalizes AT1R and p65 NF-κB protein levels following stimulation with Ang II. Values are expressed as a ratio of protein to GAPDH and normalized to no ligand. A, AT1R. B, p65 NF-κB. C, GRK5. **P*<0.05 versus no ligand; n=5 to 7. From Haack et al[79].

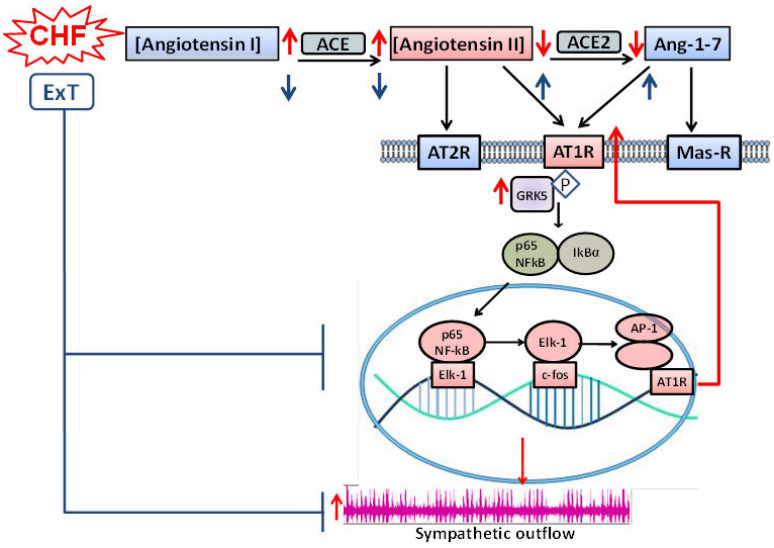



**Figure 8.**

Summary schematic showing the relationship between RAS metabolites, the AT1R signalling cascade, and sympathetic outflow in CHF and following ExT. CHF, chronic heart failure; ExT, exercise training; AP1, Activator Protein 1; NFkB, Nuclear Factor kappa B; ELK1, ETS Like Kinase; ACE, Angiotensin Converting Enzyme. In neurons Ang II stimulates the up regulation of the AT1R by an NFkB initiating process. ACE is increased and ACE2 decreased resulting in an imbalance between Ang II and Ang 1-7. ExT restores this imbalance and reduces AT1R signalling by increasing ACE2 and reducing Ang II.