The idea that activation of the sympathetic nervous system is part and parcel of the apparent positive feedback cycle of cardiac deterioration in the setting of chronic heart failure (CHF) is not a new one. Several reviews have clearly summarized the evidence for this concept using both clinical data and information from experimental models.1–4 Evidence from both neural recordings and measurement of circulating catecholamines clearly document a central origin for much of the sympathetic activation in CHF.5–7 Furthermore, the fact that sympathoinhibitory reflexes are depressed in CHF has been well established in humans8,9 and experimental animals,10–12 It has been suggested that abnormal arterial baroreflex function is an early phenomenon in CHF.13 However, experimental evidence in sino-aortic denervated dogs has challenged that concept.14 On the other hand, recent data from this laboratory has shown that depression of arterial baroreflex function is an early phenomenon in conscious rabbits during the development of pacing-induced CHF.15

In the past several years, the concept has emerged that, in addition to the depressed baroreflex, an increase in the sensitivity of several sympathoexcitatory reflexes also contribute to the sympathetic activation in CHF.16–19 Enhanced input from peripheral chemoreceptors16,20 and from receptors in the heart that traverse sympathetic pathways21 may provide for a positive feedback mechanism that exacerbates the sympathoexcitatory process, although in humans this issue is less clear.22 Activation of and enhancement in the sensitivity of these latter reflexes are attributable, in part, to changes in the interstitial milieu in which the afferents reside. Alterations in the local production of substances such as bradykinin, NO, prostaglandins, and so forth, have all been implicated as excitatory substances for cardiac and chemoreceptor afferents23–29 in heart failure.

Because it is generally well accepted that the above reflexes are abnormal in the setting of CHF, this review will concentrate on the mechanisms by which central sympathetic outflow is regulated in the CHF state and how this may impact on cardiovascular reflex function. In addition, we will comment on some new strategies that may have important therapeutic implications for patients with CHF.

Central Angiotensin II and Sympathetic Outflow in CHF

There is certainly no doubt that the central nervous system is endowed with all of the components of the renin–angiotensin II (Ang II) system (including angiotensin receptors) and that both the central and peripheral renin–Ang II systems are activated in the setting of CHF.30–36 It has been clearly demonstrated that Ang II is a sympathoexcitatory peptide with effects at several different loci that include the hypothalamus and medulla, the spinal cord, sympathetic ganglia, and at sympathetic nerve endings in peripheral tissues.37 In experiments carried out more than a decade ago, Kumagai and Reid38 clearly showed that systemic administration of the Ang II type 1 (AT1) receptor antagonist losartan inhibited the pressor and renal sympathoexcitatory responses to carotid occlusion in normal conscious rabbits. They concluded that Ang II contributed to the sympathoexcitatory response during carotid occlusion. Furthermore, Ang II inhibits baroreflex function both acutely and chronically.39,40 It is still unclear whether Ang II inhibits baroreflex function by augmenting sympathetic outflow or by playing a more central role in the baroreflex pathway. In addition to the baroreflex, Ang II mediates alterations in other cardiovascular reflexes.41–43

Both intracerebral and intravertebral injection of Ang II increases sympathetic outflow and activates various cardiovascular end points, such as arterial pressure and cardiac output.43–45 Because Ang II plays a critical role in the modulation of sympathetic responses to a variety of different reflexes, it may play a role in altering the sensitivity of sympathetic motor neurons such as those activated in the rostral ventrolateral medulla (RVLM). Of course there is no question that this peptide has excitatory effects in other important integrative and afferent pathways.46–49

Brain selective overexpression of the AT1 receptor results in an enhanced pressor response to intracerebroventricular injection of Ang II in conscious mice.50 In experimental animals, CHF markedly elevates AT1 receptor expression in various areas of the central nervous system that regulate sympathetic outflow.15,51 A study from this laboratory clearly demonstrated that AT1 receptor expression is increased in the RVLM51 and in the nucleus tractus solitarius (NTS) (Figure 1A and 1B)52 in animals with CHF. Furthermore, AT1
receptor antisense oligonucleotide administration into the cerebral ventricles reduced baseline sympathetic nerve activity in rats with CHF while having no effect in normal rats.36 Chronic infusion of Ang II directly into the central nervous system of normal animals results in sympathoexcitation and an upregulation in AT1 receptors.40 As discussed in more detail below, the mechanism by which Ang II activates central sympathetic outflow is complex, but apparently it is mediated, at least in part, by reactive oxidant stress (ROS).51,53,54

It is of interest that, at least in the central nervous system, it seems that high levels of Ang II peptide evoke an upregulation in AT1 receptors. This is in contrast to many nonpeptide receptors, which show the opposite response. The mechanism for this receptor upregulation is not well understood but can be demonstrated in other systems.55 Activation of specific transcription factors that mediate upregulation of the AT1 receptor message and protein may also be operative through the c-Jun/JNK/c-fos pathway.56 It is worth noting that chronically high levels of Ang II regulate membrane potential by altering the expression of potassium channel proteins and potassium currents.57–59 This mechanism may be very important in the setting of various disease states that are characterized by sympathoexcitation, such as CHF and hypertension. Data from the laboratory of Summers and coworkers60,61 have confirmed this mechanism. Preliminary data from our laboratory indicates that rats with CHF express lower amounts of the KV4.3 protein in the RVLM than do sham rats.

Finally, the central interactions between the AT1 and AT2 receptors may be of importance in the modulation of sympathetic outflow. Based on binding experiments, AT1 receptors are dense in the organum vasculosum of the lamina terminalis and subfornical organ, as well as in the area postrema and NTS of both WKY and SHR.62,63 Functional interactions between AT1 and AT2 receptors at the single neuron level seem to be important in regulating neuronal membrane potential. Gelband et al64 showed an interaction in the modulation of potassium channel function in cultured catecholaminergic neurons. AT receptors mediate a decrease in outward K+ current, whereas AT2 receptors do just the opposite. From the standpoint of sympathetic regulation, the ability of AT2 receptors to activate the NO pathway may be very important, especially after cellular injury.65–67 In preliminary data from this laboratory, we observed an upregulation of both AT1 and AT2 gene expression in cultured neurons exposed to Ang II. This phenomenon can also be seen in the RVLM of intact rabbits with and without CHF (Figure 2). Therefore, the participation of Ang II in the sympathoexcitatory process in the CHF state depends not only on the amount of peptide available but on the density of both AT1 and AT2 receptors at specific sites in the medulla and hypothalamus and on their modulation of ion channel function and thereby neuronal excitability.

**Figure 1.** AT1 receptor protein (A) and message (B) from the RVLM of sham and CHF rabbits (with permission, *Circulation Research*).

**Figure 2.** Western blots from the RVLM of both AT1 and AT2 receptor protein in sham and CHF rabbits. Both receptor subtypes were increased in the CHF state.

**ROS and Sympathetic Outflow in Heart Failure**

Because of the now well-accepted idea that ROSs mediates a wide variety of acute and chronic biological effects in the cardiovascular system,68 their role in mediating alterations in autonomic outflow in disease states has become of interest. Antioxidant therapy in experimental animals and in humans has been shown to normalize cardiovascular and neuronal function in various disease models.69–77 Zanzinger and Czachurski78 demonstrated that superoxide dismutase (SOD) injected into the RVLM decreased sympathetic nerve activity in swine. Several groups have now shown that ROSs stimulate central sympathetic outflow in...
outflow.79,80 Perhaps more importantly, the link between the generation of central ROS and Ang II stimulation has been supported by numerous studies in normal and pathological states. The initial demonstration that Ang II could stimulate a NAD(P)H oxidase–dependent generation of superoxide in vascular smooth muscle81 has prompted multiple studies investigating this issue in the central nervous system in both normal and disease states. Several groups have now established a link among Ang II, the AT1 receptor, and superoxide signaling in the central nervous system relating to the regulation of sympathetic nerve activity.53,80,82–84 Zimmermann et al52,82 convincingly demonstrated a requirement for superoxide in the central response to Ang II in the mouse.

In studies conducted in this laboratory, we determined the role of Ang II, the AT1 receptor, NAD(P)H oxidase, and superoxide anion on renal sympathetic nerve activity (RSNA) and arterial baroreflex function in conscious rabbits with pacing-induced heart failure.15,40,51,53 These studies document a chronic upregulation of AT1 receptors along with various subunits of the NAD(P)H oxidase complex in the RVLM. Furthermore, using both dihydroethidium staining and lucigenin chemiluminescence, we demonstrated enhanced superoxide production in the RVLM of rabbits with CHF.51 Importantly, the response to central administration of Ang II, which was augmented in CHF rabbits, could be restored to near normal by either the SOD mimetic tempol or the inhibitor of NAD(P)H oxidase, apocynin (Figure 3).40,51 Preliminary studies from our laboratory have also shown that both CuZn SOD and Mn SOD synthesis are reduced in the RVLM of rabbits with pacing-induced CHF.85

How might superoxide mediate an increase in sympathetic outflow by central neurons in CHF? It has been demonstrated by many laboratories that NO is an important sympathoinhibitory substance that is released at several sites in the brain, which regulate sympathetic function.86–91 Certainly NO can modulate neuronal activity in the RVLM, NTS, and hypothalamus via a GABA mechanism.92–95 We have shown a decrease in neuronal NO synthase (nNOS) synthesis in the central nervous system of rats and rabbits with CHF.96–98 A similar finding for eNOS in the peripheral circulation has been known for some time in both animals and humans.99–104 In addition to a reduction in the synthesis of NO, any formed NO may be immediately converted to peroxynitrite if the level of superoxide generation is high. Because NO is a well-known sympathoinhibitory substance, a reduction in bioavailable NO would predispose local neurons in the hypothalamus and medulla to become more excitabile and thereby generate an enhanced sympathetic outflow. Superoxide anion has also been shown to modulate calcium channel function in the central nervous system.105 This mechanism may contribute to neuronal excitability in the setting of CHF.

Additional evidence for a role of central NO, ROS, and AT1 receptors in the sympathoexcitatory process in both CHF and hypertension comes from experiments using 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) to modulate autonomic function. Statins have been shown to be beneficial in the treatment of both hypertension and CHF.106–110 There is clear evidence that many of the vascular pleiotropic effects of statins are mediated by their ability to augment endothelial NO synthase (NOS) and NO production.111–113 In addition, statins play a role in reducing oxidant stress and Ang II signaling.114–117 However, there is much less information on the role of statins in the central nervous system, especially with regard to their potential for altering sympathetocentric function. Studies by Kishi et al118–121 have clearly shown an upregulation of various NOS isoforms in the NTS and RVLM of stroke-prone hypertensive rats after statin treatment. These investigators further showed that inhibition of the small GTP binding protein Rho and Rho kinase mimicked many of the cardiovascular and neural effects of statin treatment.122–127 Inhibition of Rho kinase is one of the target actions for statins,128–130 Because inhibition of Rho kinase is associated with increased levels of NOS,128,131 it is plausible that statins may inhibit sympathetic outflow in NO-depleted states, such as CHF. We investigated the effects of simvastatin treatment on sympathetic function in rabbits with CHF.132,133 Statin treatment clearly reduced RSNA and enhanced baroreflex function in CHF, while having no effect in normal animals. These effects occurred in the absence of any effect on plasma lipids. Furthermore, statin treatment increased heart rate variability and restored cardiac sympathovagal balance in the CHF state. The mechanism for these effects is apparently because of a reduction in oxidative stress in the RVLM as a result of a reduction in both AT1 receptor expression and NAD(P)H oxidase production.53

To summarize, the bulk of existing data strongly indicates that Ang II plays a pivotal role in the sympathoexcitatory process in the CHF state. This peptide initiates a positive feedback mechanism by which its own receptor is upregulated. The stimulation of sympathetic outflow by Ang II is further mediated by the production of superoxide anion through the action of NAD(P)H oxidase. Neuronal activation because of an increase in calcium entry and a chronic downregulation of outward potassium currents also contributes to the effects of Ang II on sympathetic function.
Summary and Conclusions
Until recently, the mechanisms by which sympathetic activation occurs in the CHF state have been largely unknown, and therapy targeting the sympathetic nervous system and the renin–angiotensin system has been more empirical than evidence based. With the advent of newer techniques to localize and evaluate the function of the central renin–angiotensin system in normal and disease states, a picture is emerging that implicates Ang II and its receptors as a pivotal regulator of sympathetic function by virtue of its role in the generation of oxidative stress and the regulation of membrane ion channel function. One of the most important and reproducible aspects of the role of Ang II in CHF relates to the modulation of the AT₁ receptor. A novel pathway that has come from this work is the upregulation of the AT₁ receptor in the face of elevated levels of Ang II. This seems to be mediated by activation of the transcription factor AP1 through a cJun/Jnk pathway.

The increase in central oxidative stress seems to play a major role in activation of sympathetic outflow in CHF. Superoxide anion production along with a reduction in SOD protein and activity may contribute to either activation or sensitization of specific populations of neurons through alterations in both potassium and calcium channel activity. In addition, reductions in the sympathoinhibitory influence of NO because of both increased scavenging of NO and a reduction in nNOS activity in the CHF state contribute to sympathoexcitation. Figure 4 provides a schematic overview of the relevant central events and pathways that may impact sympathetic nerve activity in the CHF state and the effects of exercise training.

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