Brain mechanisms of sympathetic activation in heart failure: Roles of the renin-angiotensin system, nitric oxide and pro-inflammatory cytokines (Review)

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Abstract. Patients with chronic heart failure (CHF) have an insufficient perfusion to the peripheral tissues due to decreased cardiac output. The compensatory mechanisms are triggered even prior to the occurrence of clinical symptoms, which include activation of the sympathetic nervous system (SNS) and other neurohumoral factors. However, the long-term activation of the SNS contributes to progressive cardiac dysfunction and has toxic effects on the cardiomyocytes. The mechanisms leading to the activation of SNS include changes in peripheral baroreceptor and chemoreceptor reflexes and the abnormal regulation of sympathetic nerve activity (SNA) in the central nervous system (CNS). Recent studies have focused on the role of brain mechanisms in the regulation of SNA and the progression of CHF. The renin-angiotensin system, nitric oxide and pro-inflammatory cytokines were shown to be involved in the abnormal regulation of SNA in the CNS. The alteration of these neurohumoral factors during CHF influences the activity of neurons in the autonomic regions and finally increase the sympathetic outflow. The present review summarizes the brain mechanisms contributing to sympathoexcitation in CHF.

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1. Introduction

With the increasing morbidity of coronary heart disease and hypertension, the prevalence of chronic heart failure (CHF) has been increasing over the past several decades (1). CHF is the terminal state of various cardiovascular diseases and the mortality rate remains high in spite of the large number of studies which have been performed to clarify its underlying mechanisms and to improve its treatment (2). The most significant hallmark of CHF is the continuous interaction between the underlying myocardial dysfunction and the compensatory neurohumoral mechanisms (3). Activation of the sympathetic nervous system (SNS) is a major compensatory mechanism in the development of CHF, which may be due to the changes in peripheral baroreceptor and chemoreceptor reflexes, chemical mediators that control sympathetic outflow and central integrative sites (4). Recent studies have indicated that a variety of agents, including angiotensin II (AngII), nitric oxide (NO) and pro-inflammatory cytokines are involved in the regulation of sympathetic nerve activity (SNA) in the central nervous system (CNS) during CHF (3). The interaction between these agents and neurotransmitters can influence the neuronal activity via central autonomic pathways in different autonomic regions in the CNS. The abnormal sympathoexcitation in the CNS deteriorates the cardiac function during CHF and contributes to disease progression.

2. Main sympathetic activity-regulating nuclei in the CNS

The paraventricular nucleus (PVN) in the hypothalamus, the rostral ventrolateral medulla (RVLM) and the nucleus tractus solitarius (NTS) are three important regions within the CNS controlling SNA (5). The NTS receives direct input from the cardiopulmonary afferents, such as baroreceptors and chemoreceptors, and the area postrema (AP), and has an indirect effect on the neuronal activity of the RVLM through modulating the caudal ventrolateral medulla (CVLM) region (6,7). In addition, the NTS has neural connections to the PVN, a major integrative nucleus that can influence SNA and extracellular fluid volume, and the intermediolateral (IML) column of the spinal cord (5,8). The PVN receives signals from the sub-fornical organ (SFO), which positively influences the

activity of the PVN (9). The RVLM has a key role in determining the tonic and reflex control of SNA (10). It receives input signals from the PVN and subsequently communicates with the IML, which is also under direct influence of the PVN (11-13). Finally, the IML receives input signals from these sympathetic activity-regulating nuclei and stimulates the sympathetic ganglionic neurons projecting to target organs.

3. RAS

Previous studies have demonstrated that the renin-angiotensin system (RAS), whose main effective molecule is AngII, is a key regulator of cardiovascular system function and fluid homeostasis. RAS is activated upon decreases and inhibited upon increases of the blood pressure, increases of sodium delivery to the macula densa and volume overload. AngII has two types of receptor: AngII type 1 (AT1) receptor is responsible for water and sodium retention, aldosterone secretion and vasoconstriction, while AT2 receptor is able to dilate the blood vessels.

According to the classic mechanism, AngII exerts its effects via the circulating system. For example, the concentration of AngII in plasma is increased under CHF conditions, which can inhibit the sympathoinhibitory baroreflex and enhance the sympathoexcitatory chemoreflex (14,15). Apart from these actions, circulating AngII can also increase SNA in the CNS through binding to its AT1 receptors in certain autonomic regions, which have no blood-brain barrier, such as AP and SFO (16,17). However, a large number of studies have demonstrated the presence of an endogenous brain RAS within the CNS (18). This is supported by the fact that angiotensinogen, renin and angiotensin-converting enzyme (ACE) are generated by glial cells and neurons in a number of nuclei (19-21).

Sympathoexcitatory effect of AngII in CHF. Microinjection of AngII into the PVN was shown to increase plasma norepinephrine, renal sympathetic nerve activity (RSNA), mean arterial pressure (MAP) and the heart rate (HR) to a greater extent in rats with ischemia-induced CHF compared with those in sham rats, whereas inhibition of AT1 receptors produced a significant decrease of RSNA, MAP and HR (22). Administration of AngII into the RVLM evoked sympathoexcitation in sham rats as well as rats with CHF, with a significantly larger response in the animals with CHF (23). In addition, basal SNA and the cardiac sympathetic afferent reflex were shown to be decreased by bilateral NTS injection of the AT1 receptor antagonist losartan in rats with CHF but not in sham rats, suggesting that activation of the AT1 receptor in the NTS can increase SNA during CHF (21). Over-activity of the RAS of the endogenous brain may be partly attributed to the increased expression of AT1 receptor and ACE in the CNS. In CHF, the activity of ACE in the PVN is increased and the expression of AT1 receptor is also increased in the neurons of the PVN, RVLM and NTS (23-26). Further investigation showed that AngII-triggered mitogen-activated protein kinases (MAPKs) have an important role in the upregulation of AT1 receptor in the PVN in rats with CHF (26). Under normal conditions, AT1 receptor is weakly expressed in astrocytes, while it is upregulated in brainstem astrocytes and has a key role in enhancing

sympathetic outflow under CHF conditions. However, the expression of AT1 receptor in astrocytes of other sympathetic activity-regulating nuclei during CHF requires further study (27). In an ovine model of CHF, central infusion of losartan significantly decreased the elevated levels of cardiac sympathetic nerve activity (CSNA), while RSNA was neither elevated nor affected by losartan. Furthermore, the expression of AT1 receptor was increased in the PVN but decreased in the NTS (28). These results were not completely consistent with the observations made in rodent models of CHF and the reasons for these discrepancies remain elusive. In spite of these discrepancies, the abovementioned studies suggested that increased AngII signaling, via activating the AT1 receptor, is responsible for the enhanced SNA during CHF.

Sympathoinhibitory effects of the AT2 receptor. In contrast to the AT1 receptor, the AT2 receptor has an opposite role in regulating SNA within the CNS. Intracerebroventricular injection of AngII in AT2 receptor gene knock-out mice resulted in a greater increase in blood pressure compared to that in wild-type mice (29). Intracerebroventricular infusion of Compound 21, a non-peptide AT2 receptor agonist, decreased norepinephrine excretion and blood pressure via the NO signaling pathway in the PVN of normal rats and Compound 21 also suppressed the sympathetic outflow by improving baroreflex sensitivity in rats with CHF (30,31). Overexpression of AT2 receptor in the RVLM led to a significant decrease in MAP and urinary noradrenaline excretion in normal rats (32). However, the expression of AT2 receptor in the RVLM of CHF rats was significantly lower than that in sham rats. These studies indicated that AT2 receptor activation within the CNS causes a decrease in SNA and that impairment of AT2 receptor signaling is involved in the sympathoexcitation in CHF (23). While the exact underlying mechanisms for the beneficial effects of AT2 receptor activation have yet to be elucidated, it has been indicated that activation of the AT2 receptor may inhibit the neuronal excitability and decrease the sympathetic outflow through increasing the potassium current in neurons (33). However, direct evidence for the sympathoinhibitory effects of AT2 receptor activation in the NTS and PVN on the modulation of SNA during CHF is currently lacking and the mechanisms of AT2 receptor downregulation in CHF state require further investigation.

Interaction between AngII and neurotransmitters in the regulation of SNA. Glutamate and γ-aminobutyric acid (GABA) were observed to have a role in the regulation of SNA within the CNS. The PVN contains excitatory and inhibitory neurons, which use glutamate and GABA as neurotransmitters. Microinjection of AngII into the PVN resulted in an increase in glutamatergic and a decrease in GABAergic transmission (34,35). Glutamatergic neurons also exist in the RVLM and project to the IML. Microinjection of AngII into the RVLM increased the release of glutamate in the IML, which then enhanced the sympathetic outflow to target organs (36). In addition, microinjection of AngII into the NTS potentiated GABA release through the effect of endothelial-derived NO. Elevated levels of GABA inhibit the activity of glutamatergic neurons within the NTS, which can further inhibit the GABAergic neurons in the CVLM. As the

GABAergic neurons in the CVLM have an inhibitory effect on the glutamatergic neurons in the RVLM, the activity of these glutamatergic neurons subsequently increases and potentiates the sympathetic outflow (2,37,38). Thus, the interaction between AngII, glutamate and GABA has an important role in controlling SNA.

Interaction between AngII and reactive oxygen species (ROS) in the regulation of SNA. ROS, which include oxygen ions, free radicals and peroxides, are generated during the normal metabolism of enzymes such as NADPH oxidase. They are converted by superoxide dismutase (SOD) into hydrogen peroxide and then rapidly degraded by enzymes. Accumulating evidence indicated that ROS are important mediators of AngII signaling within the CNS and are involved in the regulation of SNA (39-41). Intracerebroventricular infusion of AngII significantly increased RSNA in rabbits with CHF accompanied by increased levels of NADPH-dependent ROS in the RVLM. In addition, pre-treatment with an intracerebroventricular infusion of SOD mimetic tempol and the inhibitor of NADPH oxidase apocynin abolished the increased RSNA induced by AngII, suggesting that ROS mediates the sympathoexcitation of AngII (42). Although the exact mechanisms by which AngII induces ROS generation have remained elusive, it has been reported that application of AngII caused NADPH-dependent ROS production in NTS neurons, which was dependent on intracellular Ca^{2+} and protein kinase C (43). ROS also have an important role in AngII-induced AT1 receptor upregulation in CHF. Intracerebroventricular infusion of tempol significantly decreased AT1 receptor protein expression in the RVLM of rabbits with CHF; furthermore, tempol reversed the AngII-induced increases in AT1 receptor mRNA expression in a neuronal cell line through inhibition of the c-Jun N-terminal kinase and AP1 signaling pathways (44). Application of AngII decreases the GABAergic currents in PVN neurons, which can be abolished by treatment with SOD; in addition, ROS enhances excitatory inputs from the PVN to the RVLM through increasing glutamatergic and decreasing GABAergic transmission (Fig. 1) (35,45). Furthermore, physical exercise decreased SNA via enhancing anti-oxidant pathways and suppressing pro-oxidant mechanisms in the RVLM of rabbits with CHF (46). These studies indicated that NADPH-dependent ROS are involved in the AngII signaling pathway and contribute to the increased sympathetic outflow.

4. NO

NO, a metabolic product of the arginine metabolism catalyzed by nitric oxide synthase (NOS), is recognized as another modulator of SNA in the CNS. NOS occurs in three isoforms: Neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). nNOS has been found to be involved in the control of sympathetic outflow within all nuclei in the PVN, NTS and RVLM, while eNOS and iNOS are mainly expressed in neurons in the brainstem (47-50). Most of the previous studies demonstrated that NO generated by these NOS isoforms exerts inhibitory effects on SNA (51).

Impaired sympathoinhibitory effect of NO in CHF. As mentioned above, the baroreflex is impaired in CHF, which

contributes to sympathoexcitation. Gene transfer of nNOS into the RVLM was shown to normalize the impaired baroreflex function (52). Similarly, overexpression of eNOS in the NTS reduced urinary norepinephrine excretion in mice with CHF (53). Microinjection of nNOS anti-sense into the PVN elicited a significant pressor in normal rats, while rats with CHF were less affected, suggesting that the sympathoexcitatory effect of nNOS anti-sense is blunted in CHF. This effect of nNOS anti-sense was accompanied by decreased nNOS protein levels in the PVN and a decreased responsiveness to NO in animals with CHF (54,55). The expression of nNOS protein was demonstrated to be downregulated in the NTS and the RVLM in rats with CHF (56). Chronic administration of AT1 receptor antagonist losartan prevented the downregulation of nNOS gene expression in the PVN, NTS and RVLM during CHF (57). Thus, it is indicated that enhanced AngII signaling in CHF decreases the expression of nNOS; however, the underlying mechanisms remain elusive. It has been demonstrated that the expression of carboxy-terminal PDZ ligand of nNOS (CAPON), a protein which can interact with nNOS and prevent NO production (58), was increased, whereas the expression of nNOS was decreased in the PVN of rats with CHF (59). Furthermore, treatment of losartan abrogated the changes in expression of CAPON and nNOS in animals with CHF, but also abolished AngII-induced increases in CAPON in neuronal cells (59). These results indicated that CAPON may act as a downstream mediator in AngII-induced downregulation of nNOS during CHF (59). In addition, the expression of protein inhibitor of nNOS (PIN) is upregulated due to increased AngII levels in the PVN under CHF conditions. Binding of PIN to nNOS leads to the de-stabilization of nNOS. As a result, the inactive monomeric state of nNOS is susceptible to ubiquitination and proteasomal degradation (Fig. 1) (60). These results suggested that the sympathoinhibitory effect of NO is impaired by decreases of nNOS, which thereby contribute to sympathoexcitation during CHF.

Mechanisms of the regulation of SNA by NO. Excitatory and inhibitory neurotransmitters have been demonstrated to be involved in the mechanism by which NO regulates the SNA. Administration of NO into the PVN has been shown to increase local levels of GABA (61). NO-induced decreases in RSNA, blood pressure and HR were inhibited by blocking of the GABAergic system, which indicated that the inhibitory effects of NO within the PVN are mediated by GABA (62). However, administration of *N*-methyl-D-aspartate (NMDA) into the PVN increased RSNA, blood pressure and HR. These responses were enhanced by prior microinjection of NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA), indicating that NO inhibits NMDA-mediated increases in SNA in the PVN (63). Gene transfer of nNOS into the PVN of rats with CHF significantly enhanced the blunted changes in RSNA, blood pressure and HR in response to L-NMMA, while the response to NMDA was significantly decreased. This effect was due to gene transfer of nNOS reducing the increased NMDA receptor sub-unit NR(1) mRNA and protein expression in the PVN of rats with CHF (64). Similarly, gene transfer of eNOS into the RVLM significantly decreased MAP and HR in normal rats, coupled with an increased expression of GABA. By contrast, microinjection of GABA receptor antagonist following gene transfer
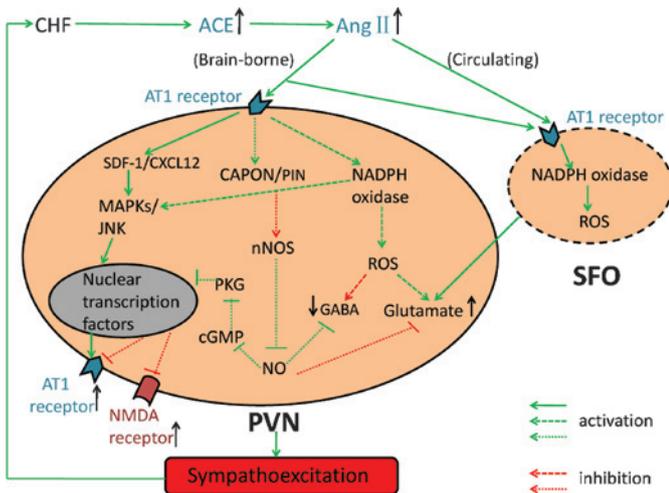
Figure 1. Effects of AngII and NO within the PVN in the regulation of SNA under CHF conditions. AngII is elevated in both peripheral tissues and CNS during CHF. The circulating AngII can bind to its AT1 receptors in the autonomic regions which have no blood-brain barrier, such as AP and SFO. The SFO has a positive influence on the activity of the PVN and activation of the SFO by AngII can increase the glutamate levels in the PVN. The brain-borne AngII is produced by multiple cell types in autonomic regions, including SFO. In the PVN, AngII increases the MAPKs, decreases the expression of NO and increases the levels of ROS. As a result, the expression of AT1 receptor is upregulated and the balance between the glutamatergic and GABAergic systems is disturbed. Finally, the action of AngII in the different autonomic regions produces sympathoexcitation under CHF conditions. AngII, angiotensinII; AT1 receptor, AngII type 1 receptor; SDF-1/CXCL12, chemokine stromal cell-derived factor-1; MAPKs, mitogen-activated protein kinases; JNK, c-Jun N-terminal kinase; CAPON, carboxy-terminal PDZ ligand of nNOS; PIN, protein inhibitor of nNOS; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; cGMP, 3',5' guanosine monophosphate; PKG, protein kinase G; NMDA receptor, N-methyl-D-aspartate receptor; ROS, reactive oxygen species; GABA, γ-aminobutyric acid; PVN, paraventricular nucleus; AP, area postrema; SFO, subfornical organ; CHF, chronic heart failure; ACE, angiotensin-converting enzyme.
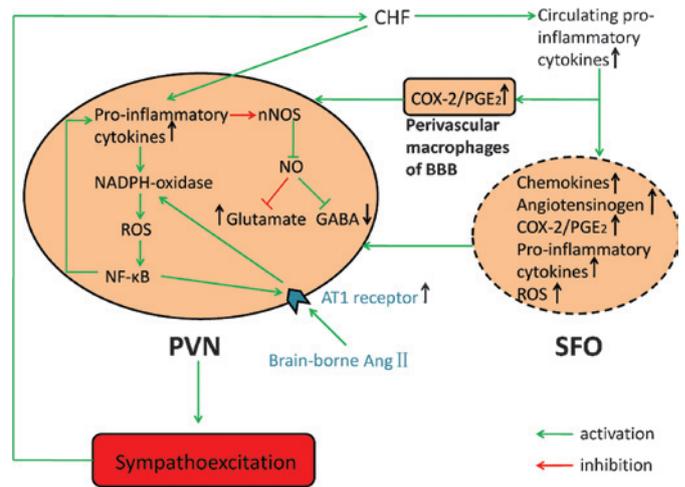

Figure 2. Effects of pro-inflammatory cytokines within the PVN in the regulation of SNA under CHF conditions. Circulating pro-inflammatory cytokines are increased during CHF, either through activation of the SFO to produce multiple neurohumoral factors or activation of perivascular macrophages to generate PGE2. These circulating pro-inflammatory cytokines can further stimulate the activity of PVN and increase the sympathetic outflow. In the PVN, the increased pro-inflammatory cytokines and AngII upregulate the expression of ROS and NF-κB via enhancing the activity of NADPH-oxidase, which in turn elevates the levels of pro-inflammatory cytokines and AT1 receptor. In addition, pro-inflammatory cytokines may also improve glutamate levels and decrease GABA levels through reducing the synthesis of NO. These alterations in the PVN finally produce sympathoexcitation during CHF. COX-2, cyclooxygenase 2; PGE2, prostaglandin E2; BBB, blood-brain barrier; ROS, reactive oxygen species; NF-κB, nuclear factor-kappaB; AT1 receptor, angiotensinII type 1 receptor; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; GABA, γ-aminobutyric acid; PVN, paraventricular nucleus; SFO, subfornical organ; CHF, chronic heart failure.

enhanced the increases in MAP (65). In addition, inhibition of iNOS in the RVLM enhanced the pressor response caused by glutamate, suggesting that NO derived from iNOS inhibited the action of glutamate in the RVLM (66). *In vitro*, NO negatively regulated AT1 receptor expression via the protein kinase G pathway in primary cultures of hypothalamic and neuronal cell lines (67), indicating that reduced NO production within the CNS during CHF may enhance the activity of the RAS (Fig. 1).

However, due to conflicting results, no consensus has been reached regarding the role of NO in the regulation of SNA in the RVLM and NTS. It has been reported that nNOS inhibition in the RVLM decreased MAP and the HR. Microinjection of nNOS inhibitor attenuated the pressor response caused by glutamate, indicating that NO derived from nNOS produces sympathoexcitation through enhancing the effects of glutamate in the RVLM (66). In addition, microinjection of L-NMMA or an nNOS inhibitor into the RVLM attenuated the cardiac sympathetic afferent reflex elicited by epicardial application of bradykinin (68). In the NTS, NO was shown to be able to potentiate GABAergic as well as glutamatergic transmission (69). However, the activation of glutamatergic transmission in the NTS evoked a decrease in blood pressure and HR (70).

It is therefore indicated that through interacting with RAS and neurotransmitters, NO produced by various isoforms of

NOS is able to decrease the SNA, while the sympathoinhibitory effect of NO is impaired in CHF. Of note, in the RVLM and NTS, NO can also increase SNA. Recently, it has been reported that endogenous NO in the CNS exerted excitatory effects to increase resting CSNA in healthy subjects, while exogenously administrated NO inhibited CSNA under normal and CHF conditions (71). Thus, the sympathoexcitatory and sympathoinhibitory effects of NO in the CNS may partly depend on the balance of glutamatergic and GABAergic activity, the specific sympathetic activity-regulating nuclei on which it acts and the method of NO administration.

5. Pro-inflammatory cytokines

Pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), are elevated in CHF and contribute to the progression of the disease (72). In analogy to AngII, peripheral pro-inflammatory cytokines can penetrate into the CNS at the nuclei where no blood-brain barrier exists; furthermore, cytokines can also be generated by multiple cell types in the CNS (73,74). Pro-inflammatory cytokines within the CNS can increase the sympathetic outflow during CHF (75).

Sympathoexcitatory effects of pro-inflammatory cytokines. Through either acting on the SFO to produce multiple neurohumoral factors or activating the perivascular macrophages to generate prostaglandin E2 (PGE2), circulating

pro-inflammatory cytokines can further stimulate the activity of the PVN and increase the sympathetic outflow (74,76). The central inflammation-induced PGE2 generation depends on the NADPH-oxidase and the p38 MAPK pathway under pathophysiological conditions (77). Furthermore, activated cardiac sympathetic afferent nerves transmit signals to the CNS to increase cytokine production, which contributes to sympathoexcitation by upregulation of the activity of the RAS and the hypothalamic-pituitary-adrenal axis in the PVN under CHF conditions (78,79). It has been indicated that nuclear factor (NF)-κB may mediate the interaction between the RAS and pro-inflammatory cytokines, as inhibition of the synthesis of NF-κB reduced cytokine and AT1 receptor expression in the PVN of rats with CHF and in turn, blockade of the AT1 receptor decreased the expression of cytokines and NF-κB (80). In addition, central TNF increased the NADPH oxidase sub-unit and ROS production in the PVN and RVLM under CHF conditions, which further activated the RAS and NF-κB (81,82). Toll-like receptor (TLR) signaling has an important role in mediating the inflammation cascade. Stimulation of TLR4 led to the activation of NF-κB via the myeloid differentiation primary-response protein 88 (MyD88)-dependent pathway, which induced pro-inflammatory cytokine production (83). Intracerebroventricular infusion of an AT1 receptor blocker decreased the elevated SNA and reduced the increased expression of TLR4, MyD88 and NF-κB in the brainstem of mice with CHF. Therefore, it is indicated that TLR4 is involved in AT1 receptor-induced pro-inflammatory cytokine production in CHF (84). Cytokines were also indicated to upregulate SNA via increasing excitatory neurotransmitters and decreasing inhibitory neurotransmitters, as intracerebroventricular infusion of cytokine blockers attenuated HF-induced increases in glutamate and decreases in GABA in the PVN of rats with CHF, probably through an NO-associated mechanism (Fig. 2) (85). In addition, chemokine stromal cell-derived factor-1 may mediate the sympathoexcitation of TNF-α and AngII in the PVN through activating p44/42 MAPK signaling, which further activates the transcription factors and upregulates the expression of AT1 receptor (Fig. 1) (86).

6. Conclusion

CHF is a clinical syndrome characterized by continuous interaction between the underlying myocardial dysfunction and compensatory neurohumoral mechanisms. Activation of neurohumoral mechanisms in the CNS contributes to the sympathoexcitation in CHF. Numerous studies have explored the role of AngII, NO and pro-inflammatory cytokines in the regulation of SNA in the CNS. By acting on sympathetic activity-regulating nuclei at various levels, these neurohumoral factors influence the activity of neurons and finally produce a sympathoexcitatory or sympathoinhibitory effect. However, the underlying mechanisms by which these factors regulate SNA remain to be fully elucidated and the interaction between these agents within the CNS also remains to be clarified. Finally, although blockade of the abnormal neurohumoral axis in the CNS has shown beneficial effects in animal experiments, further study is required to determine whether the central neurohumoral axis may represent a novel target for the treatment of patients with CHF.

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