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·Review·

Angiotensin II-derived reactive oxygen species underpinning the processing of the cardiovascular reflexes in the medulla oblongata

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Abstract: The brainstem is a major site in the central nervous system involved in the processing of the cardiovascular reflexes such as the baroreflex and the peripheral chemoreflex. The nucleus tractus solitarius and the rostral ventrolateral medulla are 2 important brainstem nuclei, and they play pivotal roles in autonomic cardiovascular regulation. Angiotensin II is one of the neurotransmitters involved in the processing of the cardiovascular reflexes within the brainstem. It is well-known that one of the mechanisms by which angiotensin II exerts its effect is via the activation of pathways that generate reactive oxygen species (ROS). In the central nervous system, ROS are reported to be involved in several pathological diseases such as hypertension, heart failure and sleep apnea. However, little is known about the role of ROS in the processing of the cardiovascular reflexes within the brainstem. The present review mainly discussed some recent findings documenting a role for ROS in the processing of the baroreflex and the peripheral chemoreflex in the brainstem.

Keywords: angiotensin II; superoxide; rostral ventrolateral medulla; nucleus tractus solitarius; baroreflex; peripheral chemoreflex

1 Introduction

The brainstem is a major integrative site in the central nervous system involved in the processing of the cardiovascular reflexes such as the baroreflex and the peripheral chemoreflex. Among its important nuclei are the nucleus tractus solitarius (NTS) and the rostral ventrolateral medulla (RVLM), which play pivotal roles in autonomic cardiovascular regulation[1]. Reactive oxygen species (ROS) have emerged as important modulators of neuronal activity in both health and disease. Although much is known about the role of ROS in the central nervous system under pathological states such as hypertension and heart failure, little is known about the role of ROS in the processing of the cardiovascular reflexes within the brainstem. In this review, some recent findings documenting a role for ROS in the processing of the baroreflex and the peripheral chemoreflex in the brainstem were discussed.

2 Integration of the cardiovascular reflexes in the brainstem

The brainstem is the main integrative center for neural control of circulation[2-4]. It receives direct input from cardiovascular afferents such as arterial baroreceptors and peripheral chemoreceptors. Arterial baroreceptors are part of the afferent arm of the baroreflex, which has a crucial role in short-term blood pressure control. Once activated, baroreceptors generate action potentials that are conducted

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to the brainstem via afferent fibers. Within the brainstem, the signals generated by the baroreceptors reach the NTS, where they make their first synapse. After reaching the NTS, 2 autonomic pathways are activated: the parasympathoexcitatory and the sympathoinhibitory pathways. In the first pathway, NTS neurons excite neurons in the dorsal motor nucleus of the vagus and in the nucleus ambiguous, areas responsible for holding the parasympathetic premotor neurons. In the second pathway, NTS neurons activate neurons in the caudal ventrolateral medulla (CVLM). Once activated, CVLM neurons inhibit the sympathetic premotor neurons in the RVLM, inhibiting their firing rate and reducing the sympathetic tone[1].

Another important cardiovascular reflex is the peripheral chemoreflex, which is a survival reflex activated mainly under severe hypoxia. Peripheral chemoreflex activation elicits intense parasympathetic-mediated bradycardia and sympatho-excitation, resulting in increases in blood pressure[5,6]. Both distinct autonomic components of the peripheral chemoreflex can be pharmacologically dissociated by blocking α-adrenergic receptors in the vessels using prazosin and blocking muscarinic receptors in the heart using atropine[7]. Therefore, upon activation, peripheral chemoreceptors generate action potentials which are conducted to the brainstem, more precisely to the NTS. It is believed that the processing of the peripheral chemoreflex within the brainstem is also composed by 2 distinct pathways: the parasympathoexcitatory and the sympathoexcitatory pathways. In the parasympathoexcitatory pathway, NTS neurons activate neurons in the nucleus ambiguous, resulting in an increase in parasympathetic discharge, increasing the vagal tone to the heart and producing a striking bradycardia, which is important for saving oxygen from the cardiac muscle metabolism and sparing this precious oxygen to the brain and kidney. The sympathoexcitatory pathway is composed of NTS neurons that project directly to the RVLM, bypassing the CVLM and exciting the presympathetic motor neurons in the RVLM, resulting in increased sympathetic drive. Upon activation, peripheral chemoreflex also elicits behavioral and respiratory responses, which are crucial for survival during severe hypoxia[8,9].

3 Neurotransmission of the cardiovascular reflexes in the brainstem

Elucidating the putative neurotransmitters involved in the processing of the cardiovascular reflexes within the brainstem has been the subject of several research groups worldwide. Several neurotransmitters have been described to be involved in the processing of the baroreflex in the NTS, such as glutamate[10,11], GABA[12], Substance P[13], angiotensin II[14], nitric oxide[15], and ATP[16]. In addition, several studies have also implicated glutamate[17], angiotensin II[18], and nitric oxide[19] in the neurotransmission of the baroreflex in the RVLM.

Regarding the peripheral chemoreflex, various neurotransmitters have been implicated in the neurotransmission of the sympathetic and parasympathetic components of the reflex in the NTS. Among them are glutamate[9], ATP[3], and angiotensin II[20]. In the RVLM, glutamate, ATP[21], angiotensin II[22], and substance P[23] are among the documented neurotransmitters. As described above, although glutamate seems to be the most important neurotransmitter for the integration of the cardiovascular reflexes within the brainstem, angiotensin II still plays an important role in both the NTS and the RVLM in order to mediate/modulate the neurotransmission of the baroreflex and the peripheral chemoreflex.

4 Angiotensin II-derived ROS underpinning the neurotransmission of the cardiovascular reflexes in the brainstem

Accumulating evidence has suggested that the key mechanism through which angiotensin II influences blood pressure is via its ability to activate ROS signaling pathways. The pathways of ROS production in mammalian cells have been reviewed[24]. The first evidence that angiotensin II activates an NADPH oxidase in vascular smooth muscle cells to produce ROS was presented by Griendling and colleagues[25]. More recently, our studies and other findings also suggest that, like vascular cells, neurons also require ROS to carry out crucial functions related to the central control of blood pressure[22,26-28].

There is compelling evidence that superoxide anion, the most important ROS, is essential for eliciting the vasopressor, bradycardiac, and dipsogenic responses produced by intracerebroventricular administration of angiotensin II in conscious mice[26]. It has also been described that angiotensin II causes robust increases in superoxide production in cultured subfornical organ neurons. In addition, adenoviral-mediated delivery of cytoplasmically targeted superoxide dismutase selectively to the subfornical organ abolishes the cardiovascular and dipsogenic actions of angiotensin II in normotensive mice and prevents the hypertension in mice with chronic peripheral angiotensin II infusion[26,29]. In addition, by using adenoviral vectors encoding small interfering RNA that selectively silences Nox2 or Nox4 (2 isoforms of the NADPH oxidase) expression in the subfornical organ, researchers show that both Nox2 and Nox4 are required for the full vasopressor effects of brain angiotensin II[30].

Of note, angiotensin receptors, mainly AT1 receptor subtype, are also present in the RVLM[31-33] and play important roles in altering the activity of RVLM neurons[34]. For example, injection of angiotensin II into the RVLM of cat produces pressor response[35]. In addition, pharmacological blockade of AT1 receptors attenuates the pressor response to angiotensin II microinjection into the RVLM of rats[36]. Furthermore, microinjection of losartan (an angiotensin II receptor antagonist) into the RVLM attenuates the pressor response produced by peripheral chemoreflex activation[22].

Despite the numerous studies involving hypertension and ROS in the central nervous system, little is known about the role of ROS in the processing of the cardiovascular reflexes within the brainstem. To date, our laboratory and others have shown that accumulation of angiotensin II-derived superoxide anions in the brainstem are critical for the impairment of both baro- and peripheral chemoreflexes[22,37-40]. In rabbits suffering chronic heart failure, intracerebroventricular administration of angiotensin II induces superoxide accumulation in the RVLM. Administration of losartan reduces ROS accumulation in the RVLM and improves baroreflex sensitivity. In addition, chronic intravascular administration of vitamin C, a well-known antioxidant capable of crossing the blood-brain barrier to scavenge ROS in the central nervous system, improves the baroreflex sensitivity in renovascular hypertensive rats[41]. Furthermore, we have shown that acute administration of vitamin C or acute inhibition of the NADPH oxidase using apocynin also improved the depressed baroreflex sensitivity in renovascular hypertensive rats[39]. Similarly, acute inhibition of the NADPH oxidase with apocynin or scavenging of superoxide with tiron, a superoxide dismutase mimetic, improves the baroreflex sensitivity in spontaneously hypertensive rats[42]. Within the central nervous system, administration of N-acetylcysteine or vitamin C modulates the parasympathetic component of the baroreflex in normotensive rats[41]. Therefore, angiotensin II-derived ROS play an important role in modulating the processing of the baroreflex in the brainstem.

In addition, we have also described a role for ROS in modulating the processing of the peripheral chemoreflex in the RVLM. Nunes *et al*.[22] have reported that bilateral microinjection of losartan, an AT1 receptor antagonist, into the RVLM attenuates the pressor and bradycardiac response elicited by peripheral chemoreflex activation in conscious rats. In addition, the bilateral microinjection of tempol, a superoxide dismutase mimetic, into the RVLM blunts the pressor and bradycardiac response to either angiotensin II or peripheral chemoreflex activation, suggesting that angiotensin II-derived ROS also play a role in the processing of the peripheral chemoreflex activation within the RVLM.

5 Conclusion

Although the role of ROS in the central nervous system under pathological states such as hypertension has been revealed, little is known about the role of ROS in the processing of the cardiovascular reflexes within the medulla oblongata. Inflammatory T cells are emerging as new participants in this complex puzzle, especially in the peripheral organs[43]. However, elucidation of the relation between ROS and inflammation in the brainstem requires further investigation. In addition, although the antioxidant therapy has shown effectiveness in ameliorating hyper-

tension in several experimental models, its use in clinical studies is still controversial[43,44]. Here we highlighted the most recent findings of our laboratory and others in order to shed some lights into this field. Considering that those cardiovascular reflexes are altered in several pathological states such as hypertension, heart failure and obstructive sleep apnea, revealing the mechanisms underpinning the processing of those reflexes within the brainstem in health and disease will help to find new therapeutic targets in the future.

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血管紧张素 II 诱导产生的活性氧簇参与延髓的心血管反射

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摘要：脑干是中枢神经系统中的一个重要部位，参与心血管反射，例如压力感受性反射和外周化学感受性反射。孤束核和延髓头端腹外侧是脑干中重要的两个部位，在心血管自主调节中扮演重要角色。神经递质血管紧张素II能通过活化一些通路，诱导产生活性氧簇，进而参与脑干心血管反射。研究表明，在中枢神经系统中，活性氧簇与一些病理疾病相关，例如高血压、心衰竭和睡眠性呼吸暂停。然而，活性氧簇在脑干心血管反射中的作用目前尚不明确。本文主要就最近关于活性氧簇在脑干中压力感受性反射和外周化学感受性反射中作用的一些发现进行综述及讨论。

关键词：血管紧张素II；过氧化物；延髓头端腹外侧；孤束核；压力反射；外周化学反射