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Research Report

Superoxide scavenging in the rostral ventrolateral medulla blunts the pressor response to peripheral chemoreflex activation

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ARTICLE INFO

Article history:
Accepted 1 July 2010
Available online 24 July 2010

Keywords:
AT_1 receptor
Free radical
Superoxide dismutase
Angiotensin-II
Hypoxia
Rat
Sensory physiology

ABSTRACT

Peripheral chemoreflex activation has been considered the key drive for the overactivity of the sympathetic nervous system observed in some pathological conditions such as sleep obstructive apnea. In addition, increases in angiotensin-II-derived reactive oxygen species found in some autonomic regulatory brain areas have been implicated in hypertension. However, a link between oxidative stress and peripheral chemoreflex integration within the RVLM has never been investigated. Here, we tested the hypothesis that the pressor response induced by peripheral chemoreflex activation involves the angiotensin-II/AT_1R/superoxide pathway within the rostral ventrolateral medulla (RVLM). Seventeen male Wistar rats (260–300 g) were implanted with bilateral guide cannulae towards the RVLM and were fitted with catheters for blood pressure recordings and drug administration. Peripheral chemoreflex activation with potassium cyanide (80 μg/kg, i.v.) produced a transient increase in blood pressure, which was attenuated 2 minutes after bilateral microinjection of losartan (1 nmol), an AT_1 receptor antagonist, in the RVLM (+54±4 vs +19±3 Δmm Hg, $P<0.05$, $n=6$). Moreover, superoxide scavenging in the RVLM using a superoxide dismutase (SOD) mimetic, Tempol (5 nmol), significantly blunted the pressor response to peripheral chemoreflex activation (+50±3 vs +18±3 Δmm Hg, $P<0.05$, $n=7$). On the other hand, bilateral microinjection of saline ($n=4$) in the RVLM produced no change in the pressor response to chemoreflex activation. Taken together, these data suggest that the neurotransmission of the peripheral chemoreflex within the RVLM involves, at least in part, the activation of AT_1 receptors and downstream superoxide formation.

1. Introduction

During systemic hypoxia, activation of peripheral chemoreceptors located in the carotid bodies leads to behavioral, respiratory, and autonomic adjustments that function to restore oxygen levels to the normal physiological range. In conscious rats, the autonomic responses to peripheral chemoreflex activation consist of elevation in blood pressure mediated by an increase in sympathetic drive to the vasculature, as well as bradycardia mediated by a predominance of parasympathetic over sympathetic drive to the heart (Franchini and Krieger, 1993; Braga et al., 2007).

In recent years, we and others have attempted to understand the neurotransmission of the peripheral chemoreflex

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doi:10.1016/j.brainres.2010.07.001

within the central nervous system (Franchini and Krieger, 1993; Koshiya and Guyenet, 1996; Guyenet, 2000; Paton et al., 2001; Braga et al., 2006a; Braga and Machado, 2006; Reddy et al., 2007; Cruz et al., 2008). In this regard, several neurotransmitters such as glutamate, ATP, serotonin, noradrenaline, GABA, neuropepitide Y, and others have been suggested to play a role in this complex neurotransmission that leads to autonomic changes via activation of different cardiovascular regulatory brain areas such as the paraventricular nucleus of the hypothalamus (Reddy et al., 2007), the nucleus tractus solitarius (Franchini and Krieger, 1993; Paton et al., 2001; Braga et al., 2006a,b), and the rostral ventrolateral medulla (Cruz et al., 2008; Mauad and Machado, 1998) in response to peripheral chemoreflex activation.

Amongst the neurotransmitters involved in the autonomic regulation in different levels of the central nervous system is angiotensin-II (Ang-II). In this context, neurons within the RVLM contain a high density of Ang-II type 1 (AT_1) receptors (Hu et al., 2002), and there is evidence that tonic activation of these receptors contributes to increased sympathetic vasomotor activity in some forms of hypertension (Fontes et al., 1997; Tagawa et al., 2000). Interestingly, in normotensive animals, blockade of AT_1 receptors in the RVLM has little effect on baseline cardiovascular function (Head and Mayorov, 2001; Bergamaschi et al., 2002).

Abundant evidence indicates that a key mechanism through which Ang-II influences blood pressure is the production of NADPH-oxidase-derived reactive oxygen species (ROS) such as superoxide ($O_2^{\cdot-}$) in the central nervous system (Zimmerman et al., 2004; Zimmerman and Davisson, 2004; Gao et al., 2004; Chan et al., 2005; Peterson et al., 2006; Han et al., 2007). Previous reports have established that ROS are important signaling intermediates in the cardiovascular effects elicited by Ang-II when it is administered either acutely and directly into the central nervous system (Zimmerman et al., 2002) or chronically as peripheral subcutaneous infusion (Zimmerman et al., 2004; Gao et al., 2005; Braga, 2010). Although Ang-II has been implicated in the processing of neuronal inputs within the RVLM affecting sympathetic outflow, a link amongst Ang-II, oxidative stress and the neurotransmission of peripheral chemoreflex within the RVLM has never been investigated.

Considering the importance of the RVLM in blood pressure regulation and modulation of sympathetic outflow, along with evidence showing that ROS mediate Ang-II signaling within the brain, we hypothesized that oxidative stress in the RVLM plays an important role in the neurotransmission of the pressor response elicited by peripheral chemoreflex activation.

2. Results

2.1. *Baseline values and cardiovascular responses to peripheral chemoreflex activation*

Baseline values for blood pressure and heart rate were 104±3 mm Hg and 346±9 bpm, respectively ($n=17$). In all animals, peripheral chemoreflex activation elicited a rapid and transient increase in blood pressure and bradycardia (ΔMAP=+51±3 mm Hg; ΔHR=−198±8 bpm; $P<0.05$ when compared to baseline values, $n=17$), while injection of saline (i.v.) in the same animals produced negligible effects on both parameters (ΔMAP=+2±2 mm Hg; ΔHR=−3±2 bpm; $n=17$). Bilateral microinjection of the AT_1 receptor antagonist losartan into the RVLM of conscious rats ($n=6$) did not significantly alter mean arterial pressure (103±5 vs 101±6 mm Hg) and heart rate (352±8 vs 362±11 bpm) from baseline values. In addition, bilateral microinjection of the superoxide dismutase mimetic tempol in the RVLM ($n=7$) did not alter baseline mean arterial pressure (106±4 vs 103±3 mm Hg) and heart rate (347±9 vs 351±10 bpm).

2.2. *Cardiovascular responses to angiotensin-II before and after the scavenging of superoxide in the RVLM*

To investigate a possible role for superoxide formation as a downstream mechanism in angiotensinergic neurotransmission within the RVLM, we performed unilateral microinjection of angiotensin-II before and after the microinjection of tempol, a superoxide dismutase mimetic in the RVLM. Unilateral microinjection of angiotensin-II (60 pmol) in the RVLM elicited significant increases in blood pressure and bradycardia, and these cardiovascular responses were attenuated following the microinjection of tempol (5 nmol) in the RVLM (22±3 Δmm Hg vs. 5±2 Δmm Hg and −19±6 Δbpm vs −6±3 Δbpm, respectively, $P<0.05$, $n=4$), suggesting that superoxide is involved in the pressor response produced by angiotensin-II in the RVLM (Fig. 1).

2.3. *Peripheral chemoreflex activation before and after the blockade of AT_1 receptors in the RVLM*

To investigate a possible role for angiotensinergic neurotransmission in modulating chemoreflex responses within the RVLM, we performed bilateral microinjections of losartan, an AT_1 receptor antagonist, in the RVLM. Fig. 2 shows a group of tracings from an animal representative of the group, illustrating the changes in blood pressure and heart rate in response to peripheral chemoreflex activation before and after the antagonism of AT_1 receptors the RVLM. Bilateral microinjections of losartan (1 nmol) in the RVLM significantly blunted the pressor response to peripheral chemoreflex activation at 2 (19±3 Δmm Hg vs 54±4 Δmm Hg, $P<0.05$, $n=6$) and 15 minutes (31±2 Δmm Hg vs 54±4 Δmm Hg, $P<0.05$, $n=6$) after the microinjection. In addition, chemoreflex-induced bradycardia was attenuated 2 (−69±10 Δbpm vs −209±10 Δbpm, $P<0.05$, $n=6$) and 15 minutes (−177±28 Δbpm vs −209±10 Δbpm, $P<0.05$, $n=6$) after the AT_1 receptor blockage in the RVLM (Fig. 3). As also illustrated in Fig. 2, all cardiovascular responses to peripheral chemoreflex activation were fully recovered 60 minutes after AT_1 receptor antagonism in the RVLM.

2.4. *Peripheral chemoreflex activation before and after the scavenging of superoxide in the RVLM*

To further investigate the mechanism within the RVLM by which peripheral chemoreflex activation elicits pressor response, taking into account that angiotensin-II induces the formation of reactive oxygen species via AT_1 receptor and downstream NADPH oxidase activation, we performed the scavenging of superoxide anions in the RVLM by the