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Research Report

Endothelial NOS expression within the ventrolateral medulla can affect cardiovascular function during static exercise in stroked rats

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ABSTRACT

Temporary occlusion of the middle cerebral artery (MCA) causing damage to brain tissue occurs in the majority of human stroke victims. Reflex cardiovascular responses during static exercise were attenuated following transient MCA occlusion (MCAO) and reperfusion, mediated via alteration of the neuronal nitric oxide synthase (nNOS) protein isoform within the rostral (RVLM) and caudal (CVLM) ventrolateral medulla (Ally, A., Nauli, S.M., Maher, T.J. 2005. Molecular changes in nNOS protein expression within the ventrolateral medulla following transient focal ischemia affect cardiovascular functions. Brain Res. [1055, 73-82]. We hypothesized that the endothelial NOS (eNOS) isoform within the RVLM and CVLM might also play a role in integrating cardiovascular function. Thus, we compared cardiovascular responses to static muscle contraction and eNOS expression within the four quadrants, i.e., left and right sides of both RVLM and CVLM in sham operated rats and in rats with a temporary 90-minute one-sided MCAO followed by 24 hour reperfusion. Increases in arterial pressure during a muscle contraction were attenuated in MCAO rats when compared to sham rats. Left-sided MCAO significantly decreased the expression of eNOS in the ipsilateral side but not contralateral RVLM, and to both RVLM quadrants in sham-operated rats. In contrast, compared to sham rats and the right CVLM quadrant of MCAO rats, eNOS expression was significantly increased in the left ipsilateral CVLM quadrant in left-sided MCAO rats. These data suggest that attenuation of cardiovascular responses during muscle contraction in MCAO rats may be partly due to a reduction in eNOS expression within the ipsilateral RVLM and an overexpression of eNOS within the ipsilateral CVLM. Results demonstrate that the eNOS protein within the medulla may play a significant role in mediating cardiovascular responses during static exercise in pathophysiological conditions, such as stroke.

1. Introduction

Cardiovascular diseases, including heart attack and stroke, are major causes of morbidity and mortality in U.S.A. Particularly, stroke has been ranked as the third most deadly disease in this country (Gordon et al., 2004). A proper diet, healthy lifestyle, and regular exercise are essential for the prevention of and recovery from stroke and other cardiovascular diseases (Gordon et al., 2004). The increases in mean arterial pressure (MAP), heart rate (HR), and metabolic activity observed during exercise establish and maintain a healthy circulatory system (Gordon et al., 2004). In this study, we used static exercise in anesthetized rats to elicit

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reflex cardiovascular responses. Static exercise, or isometric skeletal muscle contraction, increases MAP, HR, and sympathetic nerve activity via the contraction-induced activation of group III and group IV muscle afferents, commonly referred to as the "*exercise pressor reflex*" (Ally, 1998, Ally et al., 2002, 2005, 2006; Bauer et al., 1992; Ishide et al., 2001, 2003, 2005, Iwamoto and Kaufman, 1987; Nauli et al., 2001; Waldrop et al., 1996). These cardiovascular changes are integrated in the ventrolateral medullary (VLM) region of the brainstem (Ally, 1998, Ally et al., 2002, 2005, 2006; Bauer et al., 1992; Ishide et al., 2001; Nauli et al., 2001; Waldrop et al., 1996). The molecular and neural mechanisms within the VLM that regulate the circulatory responses to static exercise in physiological, and particularly in pathophysiological states such as stroke are not well established.

The VLM is anatomically and physiologically divided into rostral (RVLM) and caudal (CVLM) regions that play opposing roles in the overall integration of cardiovascular function and the outflow of sympathetic nerve activity during the *exercise pressor reflex* (Ally, 1998; Bauer et al., 1992; Iwamoto and Kaufman, 1987; Waldrop et al., 1996; Willette et al., 1983). Group III and IV muscle afferents, activated by exercising skeletal muscles, terminate within the VLM (Ally, 1998; Bauer et al., 1992; Iwamoto and Kaufman, 1987; Waldrop et al., 1996). Simultaneously, the RVLM and CVLM receive input from rostral and midbrain brain structures, including the paraventricular and supraoptic nuclei of the hypothalamus (Ally, 1998; Lovick, 1993; Saper et al., 1976), the Kölliker-Fuse nucleus (Ally, 1998; Ross et al., 1984), the parabrachial complex and striatum (Krukoff et al., 1993), the midbrain periaqueductal gray matter (Lovick, 1993; Waldrop et al., 1996), parts of the cortical lobes, and the insular cortex (Ross et al., 1984; Waldrop et al., 1996). These projections use glutamatergic and GABAergic pathways that are critical for the overall regulation of cardiovascular responses during exercise (Ally, 1998; Waldrop et al., 1996). The connections in the brain associated with static exercise are schematized in our previous publication (Ally et al., 2002).

Brain ischemia initiates a cascade of events: loss of ATP and subsequent cellular dysfunction; Na^{+} and Ca^{++} influx causing massive Ca^{++}-induced release of neurotransmitters such as glutamate, which results in excitotoxicity and neuronal death; cell swelling; and finally apoptosis (Choi et al., 1998; Fisher, 2001; Moro et al., 2004; Wang et al., 2001; White et al., 2000). Most importantly, neuronal death following ischemia is attributed to the progressive generation of free radicals that damages DNA, proteins and lipids, and compromises the integrity of the blood-brain barrier (Arundine and Tymianski, 2003; Fisher, 2001; Moro et al., 2004; Schulz et al., 2005). One such free radical is nitric oxide (NO), which is generated from neurons, nerve terminals, and/or the endothelium of blood vessels supplying the brain (Cardenas et al., 2000; Chan, 1996; Choi, 1993; Choi et al., 1998; Fisher, 2001; Huang et al., 1994; Wang and Pang, 1993). The production of NO following stroke is dependent upon the activation of 3 isoforms of the NOS enzyme: calcium-dependent nNOS (Type I) produced in neurons (Fisher, 2001; Fostermann et al., 1996; Moro et al., 2004; Schulz et al., 2005); calcium-independent iNOS (Type II) synthesized in glial cells (Fisher, 2001; Fostermann et al., 1996; Moro et al., 2004; Schulz et al., 2005); and eNOS (Type III) derived from endothelial cells (Fostermann et al., 1996; Moro et al., 2004; Schulz et al., 2005). nNOS-, eNOS-, and iNOS-cGMP signal transduction processes within the RVLM and CVLM regulate cardiovascular responses and central sympathetic outflow (Chan et al., 2001; Chan et al., 2001; Kishi et al., 2001; Patel et al., 2001). Overexpression of eNOS in the RVLM evokes a decrease in blood pressure and bradycardia (Kishi et al., 2001), whereas blockade of iNOS within the RVLM elicits a pressor response (Chan et al., 2001). In addition, nNOS antagonism within the RVLM elicits depressor responses and tachycardia (Chan et al., 2001). Blockade of nNOS or eNOS within the RVLM potentiates cardiovascular responses during static exercise due to an increase in glutamate and a reduction in GABA levels (Ishide et al., 2003; Ishide et al., 2005) and iNOS antagonism within the RVLM produces the opposite result (Ally et al., 2006). The effects within the CVLM are opposite to those in the RVLM; perfusion of an nNOS or eNOS inhibitor into the CVLM attenuates the *exercise pressor reflex* by reducing glutamate and increasing GABA levels (Ishide et al., 2003, 2005), whereas an increase in glutamate and a decrease in GABA are seen after the blockade of iNOS within the CVLM (Ally et al., 2006). A balance among nNOS-, eNOS-, and iNOS-driven glutamatergic and GABAergic neurotransmission appears to maintain cardiovascular homeostasis during static exercise.

Glutamate concentration within the RVLM was significantly attenuated and GABA level increased during a static muscle contraction after a transient middle cerebral artery occulsion (MCAO) with 24 hours of reperfusion; this in turn, inhibited the *exercise pressor reflex* (Ally et al., 2002). Furthermore, a left-sided MCAO with 24 hours of reperfusion significantly attenuated nNOS expression within the left (ipsilateral) RVLM relative to the contralateral RVLM (Ally et al., 2005). Simultaneously, nNOS protein expression within the ipsilateral CVLM following a left-sided MCAO was significantly increased compared to the contralateral CVLM (Ally et al., 2005). These results suggest that one-sided temporary ischemic stroke affected ipsilateral nNOS protein expression within the VLM, which in turn resulted in the attenuation of cardiovascular responses during static exercise (Ally et al., 2002). In this present study, we investigated whether a transient brain ischemic insult alters the eNOS protein expression within the RVLM or CVLM and elicits compromised cardiovascular responses during static exercise.

2. Results

2.1. *Cardiovascular responses to muscle contraction in sham and MCAO rats*

Baseline MAP and HR were not significantly different between groups (MAP: 105±6 mmHg and 112±6 mmHg; HR : 386±15 bpm and 374±13 bpm; Not significant; $p>0.05$). In sham-operated rats following 2 hours of stabilization, a 2-minute static muscle contraction significantly increased MAP, HR, and developed muscle tension by 24±4 mmHg, 27±5 bpm, and 943±45 g, respectively ($n=10$, $p<0.05$) as shown in previous studies (Ally, 1998, 2002, 2005; Bauer et al., 1992; Ishide et al., 2001, 2003, 2005, Iwamoto and Kaufman, 1987; Nauli et al., 2001; Waldrop et al., 1996). Thereafter, a second and a third muscle contraction at 1 hour intervals elicited similar changes in MAP, HR, and developed muscle tension. In MCAO rats ($n=10$), an ipsilateral static muscle contraction also changed all three parameters, but