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Brain Research 1055 (2005) 73 – 82
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research Report

Molecular changes in nNOS protein expression within the ventrolateral medulla following transient focal ischemia affect cardiovascular functions

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Accepted 29 June 2005
Available online 9 August 2005

Abstract

The majority of human strokes involve an occlusion of the middle cerebral artery and subsequent damage to the brain tissues it perfuses. We have previously reported that reflex cardiovascular changes during a static muscle contraction are attenuated following transient middle cerebral artery occlusion (MCAO) and reperfusion [A. Ally, S.M. Nauli, T.J. Maher, Cardiovascular responses and neurotransmission in the ventrolateral medulla during skeletal muscle contraction following transient middle cerebral artery occlusion and reperfusion, Brain Res. 952 (2002) 176–187]. We hypothesized that the attenuation is a result of altered expression of neuronal nitric oxide synthase (nNOS) within the rostral (RVLM) and caudal ventrolateral medulla (CVLM). In this study, we have compared cardiovascular responses and nNOS protein expression within the four quadrants, i.e., left and right sides of both RVLM and CVLM in sham-operated rats (n = 10) and in rats with a temporary 90-min left-sided MCAO followed by 24 h reperfusion (n = 10). Increases in mean arterial pressure during a static muscle contraction were significantly attenuated in MCAO rats when compared to sham rats. The transient ischemia reduced nNOS expression within the ipsilateral RVLM quadrant compared to the contralateral RVLM or RVLM quadrants of control rats. In contrast, compared to sham rats and the right CVLM quadrant of MCAO rats, nNOS expression was significantly augmented in the ipsilateral CVLM in left-sided MCAO rats. These data suggest that the attenuation of cardiovascular responses during static muscle contraction in MCAO rats is partly due to a reduction in nNOS expression within the ipsilateral RVLM and an overexpression of nNOS abundance within the ipsilateral CVLM. Results demonstrate that nNOS expression within the medulla plays a significant role in mediating cardiovascular responses during static exercise in intact and pathophysiological conditions.

Theme: Endocrine and autonomic regulation
Topic: Cardiovascular regulation

Keywords: Rostral ventrolateral medulla; Caudal ventrolateral medulla; Stroke; Autonomic regulation; Microdialysis; Blood pressure; Exercise pressor reflex

1. Introduction

Exercise increases mean arterial pressure (MAP) and heart rate (HR) in conscious humans and both awake and anesthetized animal models, including rats [1,18,26,61]. In conscious animals, exercise involves an interaction between activation of neurons within rostral brain structures and a peripheral feedback mechanism involving the contracting muscles, commonly referred to as the "exercise pressor reflex" [1,61]. However, the central site of integration of cardiovascular responses during exercise has been shown to be the ventrolateral medulla (VLM) located in the brainstem [1,61]. The VLM is also a critical site involved in maintenance of vasomotor tone, baroreceptor, cardiopulmonary reflex mechanisms [1,12,14,52,61], and vasomotor changes in response to cerebral ischemia [13].

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0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.06.087

The VLM is anatomically and physiologically divided into the rostral (RVLM; pressor area) and the caudal (CVLM; depressor area) regions, and these areas are known to play opposing roles in the overall regulation of cardiovascular activity during exercise [1,61,65]. Activation of group III and IV muscle afferents arising from exercising muscles terminates within the VLM [1,61]. Simultaneously, both the RVLM and CVLM receive input from numerous rostral and midbrain structures, including the paraventricular and supraoptic nuclei of the hypothalamus [1,38,52], the caudolateral bed of the stria terminalis [50], the Kölliker–Fuse nucleus [50], nuclei of the parabrachial complex and striatum [34], midbrain periaqueductal gray matter [5,38], the ventromedial hypothalamic areas [52], and cortex and the insular cortex [1,50,61]. Thus, afferent impulses and neural projections originating from the above rostral brain areas relayed onto the RVLM and/or CVLM neurons play a critical role in the regulation of cardiovascular responses during exercise [1,61]. A schematic diagram of ascending and descending neural connections in the brain involving the RVLM and CVLM regions and associated with static exercise is illustrated in Fig. 1.

Numerous studies have identified different models of focal cerebral ischemia where (i) the ischemic process and pathophysiological response were similar to human stroke, (ii) ischemic infarct size was reproducible, (iii) physiological variables were monitored, and (iv) brain samples were readily available for evaluating histopathological, biochemical, and molecular changes [16,22,33,64]. Because human ischemic stroke is often caused by occlusion of the middle cerebral artery (MCA), a pannecrotic focal ischemia can be induced by MCA occlusion (MCAO) in animals [33,37,40,41,45] that simulates human stroke conditions. The MCA supplies blood to several brain areas, including the insular cortex, motor cortex, frontoparietal cortex, lateral caudoputamen, and striatum: regions involved in the integration of cardiovascular functions and autonomic responses [16,51,64]. Focal ischemia initiates a cascade of molecular events, including Ca^{++}-induced GABA and glutamate release and changes in cAMP levels [7,9,10,11,16,53]. In addition, other cellular changes during stroke that may affect the cardiovascular system include generation of free radicals and nitric oxide (NO) from vulnerable neurons, peroxide formation, superoxides, apoptosis, and differential activation of various isoforms of nitric oxide synthase (NOS) [4,10,16,23,32]. Three isoforms of NOS have been identified: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) [16,17,47]. Histochemical staining studies have revealed the existence of these isoforms within the RVLM and CVLM ([8,9,15,31]; Fig. 1). Thus, critical roles of NO and NOS have been postulated in the pathogenesis of brain damage following an acute ischemic injury. The literature suggests both neuroprotective and neurotoxic effects of NOS in cerebral ischemia [16,39,45].

The linkage between focal ischemia during a transient MCAO, RVLM, and NOS expression appears casual; however, numerous studies have suggested both a direct and indirect correlational relationship that in turn might influence cardiovascular functions. In situ hybridization analysis of c-*fos* and c-*jun* mRNAs expression in the rat brain following a transient focal ischemia has implicated both RVLM and CVLM, areas far away from the region supplied by the MCA [63,66]. Retrograde localization of the innervations of the MCA with horseradish peroxidase suggests the involvement of adrenergic nerves innervating the MCA and originating from several important ipsilateral regions, including the VLM [59]. Finally, the fact that the VLM neurons are influenced by an ischemic insult to rostral areas supplied by the MCA can be further supported by their role in cardiovascular regulation during suprapontine ischemia [21], global cerebral ischemia [19],

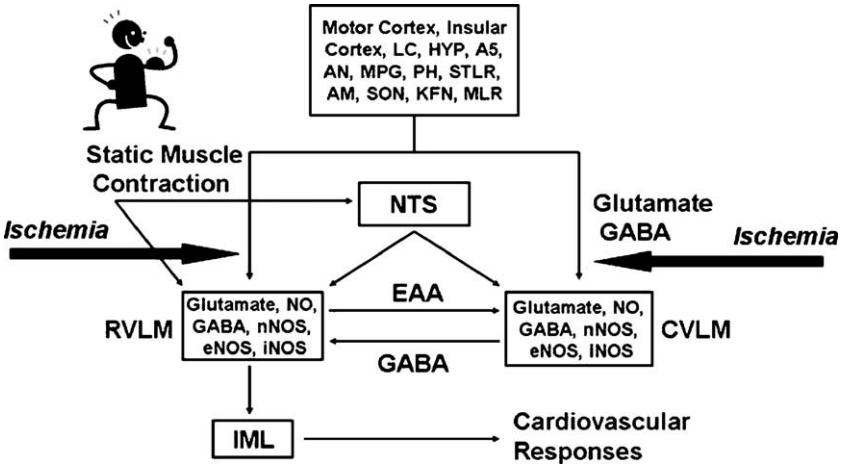

Fig. 1. A schematic diagram of brain regions and neural pathways showing major neurotransmitters involved in cardiovascular responses during static exercise. Abbreviations: AM—amygdala; AN—arcuate nucleus; CVLM—caudal ventrolateral medulla; EAA—excitatory amino acid; eNOS—endothelial nitric oxide synthase; GABA—γ-aminobutyric acid; HYP—hypothalamus; IML—intermediolateral column of the thoracic spinal cord; iNOS—inducible nitric oxide synthase; KFN—Kölliker–Fuse nucleus; LC—locus caeruleus; MPG—midbrain periaqueductal gray; nNOS—neuronal nitric oxide synthase; NO—nitric oxide; NTS—nucleus tractus solitarii; PH—posterior hypothalamus; RVLM—rostral ventrolateral medulla; SON—supraoptic nucleus; STLR—subthalamic locomotor region.