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Research Report

Cardiovascular responses and neurotransmitter changes during static muscle contraction following blockade of inducible nitric oxide synthase (iNOS) within the ventrolateral medulla

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ABSTRACT

The enzyme nitric oxide synthase (NOS) which is necessary for the production of nitric oxide from L-arginine exists in three isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Our previous studies have demonstrated the roles of nNOS and eNOS within the rostral (RVLM) and caudal ventrolateral medulla (CVLM) in modulating cardiovascular responses during static skeletal muscle contraction via altering localized glutamate and GABA levels (*Brain Res.* 977 (2003) 80–89; *Neuroscience Res.* 52 (2005) 21–30). In this study, we investigated the role of iNOS within the RVLM and CVLM on cardiovascular responses and glutamatergic/GABAergic neurotransmission during the *exercise pressor reflex*. Bilateral microdialysis of a selective iNOS antagonist, aminoguanidine (AGN; 1.0 μM), for 60 min into the RVLM attenuated increases in mean arterial pressure (MAP), heart rate (HR), and extracellular glutamate levels during a static muscle contraction. Levels of GABA within the RVLM were increased. After 120 min of discontinuation of the drug, MAP and HR responses and glutamate/GABA concentrations recovered to baseline values during a subsequent muscle contraction. In contrast, bilateral application of AGN (1.0 μM) into CVLM potentiated cardiovascular responses and glutamate concentration while attenuating levels of GABA during a static muscle contraction. All values recovered after 120 min of discontinuation of the drug. These results demonstrate that iNOS within the ventrolateral medulla plays an important role in modulating cardiovascular responses and glutamatergic/GABAergic neurotransmission that regulates the *exercise pressor reflex*.

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1. Introduction

Nitric oxide (NO), derived from L-arginine by the action of the enzyme nitric oxide synthase (NOS), is implicated in a wide range of physiological, pathophysiological, and pharmacological actions, including effects on the cardiovascular system (Krukoff, 1998; Morikawa et al., 1992; Snyder, 1992; Takeda et al., 1997; Tseng et al., 1996). Direct microinjections of NO, NO donors, or NO precursors into the rostral ventrolateral medulla (RVLM) decrease MAP and somato-sympathetic signal transmission (Shapoval et al., 1991). Systemic administration or microinjection of N_G-methyl-L-arginine (L-NMMA), a NOS inhibitor, into the RVLM increases MAP (Shapoval et al., 1991; Wang and Pang, 1993; Wang et al., 2001). Opposite effects of NO are seen if microinjected into the caudal ventrolateral medulla or CVLM (Shapoval et al., 1991). Three isoforms of NOS are characterized: (1) calcium-dependent neuronal NOS (nNOS: Type I) expressed in neurons and glial cells (Bredt et al., 1990); (2) the calcium-independent inducible NOS (iNOS, Type II) localized in macrophages, smooth muscle cells, and glial cells (Murphy et al., 1993); and (3) the endothelial NOS (eNOS, Type III) present in endothelia, platelets, and cardiomyocytes (Fostermann et al., 1995; Wong et al., 1996). Histochemical staining studies reveal the existence of these isoforms within the RVLM and caudal ventrolateral medulla (CVLM) (Chan et al., 2001b,c; Dawson et al., 1991a; Kishi et al., 2001). Furthermore, nNOS-, eNOS-, and iNOS-cGMP signal transduction processes within the RVLM and the CVLM are involved in regulation of cardiovascular responses and central sympathetic outflow (Chan et al., 2001b,c; Dawson et al., 1991a; Huang et al., 1995; Patel et al., 1996, 2001; Wu et al., 2001). For example, overexpression of eNOS in the RVLM evokes a decrease in blood pressure and bradycardia (Kishi et al., 2001), whereas nNOS antagonism within the RVLM elicits a depressor response and a decrease in HR (Chan et al., 2001c). In addition, blockade of iNOS within the RVLM elicits a pressor response (Chan et al., 2001c). Although it appears that eNOS, nNOS, and iNOS are expressed constitutively within the VLM, the physiological significance of such expression is yet to be established.

The VLM region of the brainstem is crucial in integrating increases in mean arterial pressure (MAP) and heart rate (HR) during static skeletal muscle contraction, commonly referred to as the "exercise pressor reflex" (Ally, 1998; Ally et al., 2002; Freda et al., 1999; Ishide et al., 2000a,b, 2003; Li, 2002). Both RVLM and CVLM regions are involved in cardiovascular activities and functions by integrating complex signal transduction processes, neurotransmitters, and an array of numerous neural pathways (Ally, 1998; Dampney et al., 2000; Ishide et al., 2000a, 2001, 2005; Reidman et al., 2000). Among the neurotransmitters, glutamate, an excitatory amino acid (EAA), and GABA, an inhibitory neurotransmitter, are known to play major roles in the central integration of cardiovascular activities (Ally, 1998; Somogyi et al., 1989; Sun, 1996). For example, static skeletal muscle contraction simultaneously increases the release of glutamate from neurons within the RVLM and CVLM (Ally, 1998; Ally et al., 2002; Ishide et al., 2001). The *exercise pressor reflex* also involves a baroreceptor-dependent increase in GABA concentrations within the RVLM and a decrease in GABA levels within the CVLM (Ishide et al., 2000b; Nauli et al., 2001). However, interactions of glutamate/GABA- and NO-mediated mechanisms within the VLM are the focus of much attention.

As mentioned above, NO, synthesized by the enzyme NOS, plays a critical role in cardiovascular regulation (Krukoff and Khalili, 1997; Tseng et al., 1996; Zanzinger and Seller, 1997; Zanzinger et al., 1995). Administration of L-arginine into the RVLM attenuates cardiovascular responses during static muscle contraction by reducing glutamate levels and increasing concentrations of GABA within the RVLM (Freda et al., 1999; Ishide et al., 2000a; Nauli et al., 2001). In contrast, microdialyzing L-arginine into the CVLM potentiates cardiovascular responses during static exercise via augmenting glutamate release and decreasing GABA concentrations (Freda et al., 1999; Ishide et al., 2000a). The existence of nNOS containing neurons within the RVLM that are activated during static skeletal muscle contraction is well known (Li, 2002). Our laboratory has shown that blockade of nNOS or eNOS within the RVLM augmented reflex cardiovascular responses during muscle contraction, increased glutamate levels, and decreased GABA concentrations (Ishide et al., 2003, 2005). Opposite effects are demonstrated following blockade of nNOS or eNOS within the CVLM (Ishide et al., 2003, 2005). Thus, both nNOS and eNOS modulate the *exercise pressor reflex* via altering glutamate and GABA neurotransmitter release. Although the effects of iNOS within the VLM on cardiovascular functions (Chan et al., 2001b,c), glutamate release (Perez-Asensio et al., 2005), and GABA neurotransmission (Harvey et al., 2004) are known, its role in modulating cardiovascular activity during static exercise has not been investigated.

We hypothesized that in addition to nNOS and eNOS (Ishide et al., 2003, 2005), the iNOS isoform would modulate the *exercise pressor reflex*. Therefore, the purpose of the present study is to determine the effects of iNOS antagonism within the RVLM and CVLM on cardiovascular responses during static skeletal muscle contraction and to analyze localized extracellular glutamate and GABA concentrations using microdialysis techniques. The results may confirm iNOS-glutamatergic and/or iNOS-GABAergic involvement in the neural control of cardiovascular functions during static exercise.

2. Results

2.1. *Functional integrity of RVLM and CVLM regions after insertion of microdialysis probes*

Muscle contractions were elicited before and after insertions of microdialysis probes into the RVLM or the CVLM. These contractions were performed to verify whether the probes caused tissue damage and affected the functional integrity of the regions. In every experiment before any probe was inserted, a static muscle contraction increased MAP and HR by 24 ± 3 mm Hg and 24 ± 4 bpm, respectively (n = 30). Following insertions of probes, muscle contractions increased MAP and HR by 26 ± 4 mm Hg and 27 ± 5 bpm, respectively. Thus, there was no significant damage to either RVLM or CVLM.