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Research Report

Effects of endothelial NOS antagonism within the periaqueductal gray on cardiovascular responses and neurotransmission during mechanical, heat, and cold nociception

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ABSTRACT

Nitric oxide (NO) is synthesized from L-arginine using NO synthase (NOS) enzyme that exists as 3 isoforms: endothelial (eNOS), neuronal (nNOS), and inducible (iNOS). We examined the role of eNOS within the dorsolateral periaqueductal gray mater (dlPAG) on cardiovascular responses along with glutamate and GABA concentrations during mechanical-, heat-, and cold-induced nociception in anesthetized rats. Mechanical stimulus was applied by a 10-second hindpaw pinch that increased mean arterial pressure (MAP) and heart rate (HR). Bilateral microdialysis of a selective eNOS antagonist, L-*N*(5)-(1-iminoethyl)ornithine (L-NIO; 10 μM), into the dlPAG had no effect on MAP or HR during a mechanical stimulation. Heat stimulus was generated by immersing a hindpaw metatarsus in a water-bath at 52 °C for 10 s which increased glutamate, GABA, MAP and HR. Administration of L-NIO into the dlPAG augmented cardiovascular responses and glutamate increase, but attenuated GABA changes during the heat stimulus. In contrast, application of a cold stimulus by immersing the hindpaw at 10 °C for 10 s resulted in decreases in MAP, HR, and glutamate. However, there was an increase in GABA concentration. Following microdialysis of L-NIO into the dlPAG, the responses to the cold stimulus was reversed i.e., the cold stimulus induced pressor and tachycardic responses, augmented glutamate, and attenuated GABA levels. These results demonstrate that eNOS within the dlPAG plays a differential role on the cardiovascular system during heat- and cold-mediated nociception via modulating glutamatergic/GABAergic neurotransmission. However, the mechanical stimulation had no effect on cardiovascular responses following eNOS antagonism within the dlPAG.

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1. Introduction

Nociception or pain evokes variable autonomic and behavioral responses (Zamir and Maixner, 1986; Keay and Bandler, 1993; Lovick, 1993; Bandler and Keay, 1996; Mason, 1999; Le Bars et al., 2001; Mason, 2005; Green et al., 2006). For example, cutaneous and superficial somatic pain elicit increases in mean arterial pressure (MAP), heart rate (HR), myocardial contractility, and sympathetic nerve activity; the classic "fight or flight" responses (Randich and Maixner, 1984; Zamir and Maixner, 1986; Lovick, 1993; Le Bars et al., 2001). The pain information arising from peripheral nociceptors is transmitted via myelinated (Aδ) and unmyelinated (C) fibers to different brain regions, including several cardiovascular regulatory centers, where the impulses are integrated in order to evoke the reflex autonomic responses (Randich and Maixner, 1984; Zamir and Maixner, 1986; Lovick, 1993; Le Bars et al., 2001). However, the mechanisms that coordinate the cardiovascular and autonomic reactions during pain are not fully understood.

The rostral ventrolateral medulla (RVLM) participates in the integration of pain impulses with changes in cardiovascular, autonomic and behavioral function, including localized increases in the concentration of the excitatory amino acid, glutamate, and γ-aminobutyric acid (GABA), the inhibitory neurotransmitter (Gebhart and Randich, 1990; Sun and Spyer, 1991; Lovick, 1993; Blessings, 1997; Ally, 1998; Le Bars et al., 2001). Our laboratory has shown that antagonism of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors within the RVLM attenuates the increases in MAP, HR, and glutamate levels within the RVLM during mechanical, but not in response to heat-induced thermal nociception (Gray et al., 2001). Furthermore, blockade of *N*-methyl-D-aspartate (NMDA) receptors within the RVLM attenuates cardiovascular responses by decreasing localized serotonin and dopamine concentrations during mechanical stimulation, but not in response to hot thermal stimulation (Karlsson et al., 2006). Administration of L-arginine, a precursor of nitric oxide (NO), into the RVLM inhibits cardiovascular responses to heat-induced pain by attenuating glutamatergic neurotransmission (Ishide et al., 2003). The midbrain periaqueductal gray (PAG) matter also significantly contributes to cardiovascular regulation and pain modulation (Reichling et al., 1988; Lovick, 1993, 1996; Bandler and Keay, 1996; Le Bars et al., 2001). Particularly, the lateral (lPAG) and dorsolateral (dlPAG) portions of the PAG mediate increases in MAP and HR during cutaneous and somatic nociception (Reichling et al., 1988; Lovick, 1996; Le Bars et al., 2001). We have shown that NO mechanisms within the dlPAG attenuate cardiovascular responses and concentrations of glutamate during both mechanical and heat-induced thermal nociceptive stimuli (Ishide et al., 2005).

Nitric oxide, acting via the second messenger cyclic GMP (cGMP), plays a pivotal role in coordinating the cardiovascular system (Snyder, 1992; Zanzinger and Seller, 1997; Chikada et al., 2000; Chan et al., 2001a,b). Nitric oxide is synthesized from L-arginine via the enzyme NO synthase (NOS) that exists as three isoforms: calcium-dependent neuronal NOS (nNOS) or Type I (Bredt et al., 1990); calcium-independent inducible NOS (iNOS) or Type II (Murphy et al., 1993); and endothelial NOS (eNOS) or Type III (Forstermann et al., 1995). We have shown that blockade of the nNOS protein within the dlPAG augments cardiovascular activity to heat-induced thermal, but not in response to mechanical nociception; these effects are most probably mediated by increased glutamate and attenuated GABA levels (Karlsson et al., 2007). In the present study, we focused on the eNOS isoform to determine its effect on cardiovascular responses, glutamate, and GABA neurotransmission during mechanical- and heat-induced thermal nociception. In addition, we are introducing for the first time the cardiovascular events and neurotransmission changes that follow in response to a brief 10-second cold-induced thermal stimulus and investigated the role of eNOS within the dlPAG on the cold stimulus-induced responses. Microdialysis techniques were used to administer a specific eNOS antagonist, L-N(5)-(1-iminoethyl)ornithine (L-NIO) into the dlPAG and dialysates were collected to analyze the extracellular fluid concentrations of glutamate and GABA. Overall, the purpose of the present study was to delineate the interactions between eNOS-glutamatergic and eNOS-GABAergic neurotransmission on cardiovascular function in response to mechanical, heat and cold nociception.

2. Results

2.1. *Effects of L-NIO on MAP, HR, and concentrations of GABA within the dlPAG during mechanical stimulation*

After surgical setup and insertion of microdialysis probes into the dlPAG, baseline cardiovascular parameters were stable during the 120-minute stabilization period. In addition, the rats were deeply anesthetized and there were no fluctuations of MAP or HR. Also, the rats had no corneal reflex and a pinch on the earlobe or tail did not change MAP or HR. Thereafter, a mechanical stimulation for 10 s was applied to a hindpaw and cardiovascular responses were recorded that served as controls. Then, L-NIO was bilaterally administered into the dlPAG at sequential doses of 0.1 μM, 1 μM, and 10 μM for 30 min at a rate of 5 μL/min ($n=5$). These concentrations of L-NIO did not alter baseline MAP or HR. In addition, a subsequent mechanical stimulation had no effect on the increases in MAP or HR (Fig. 1). Because a higher log dose of 20 μM L-NIO increased baseline MAP and HR, it was not used in the dose–response or any other experiment.

Thereafter, we used 8 rats in a set of experiments to determine the effects of 10 μM L-NIO on cardiovascular responses, glutamate and GABA concentrations within the dlPAG during mechanical stimulations. Mechanical stimuli increased MAP and HR by 26±4 mmHg and 36±6 bpm, respectively, that returned to pre-stimulation levels almost immediately ($n=8$; Fig. 2). Thirty minutes of bilateral administration of 10 μM L-NIO into the dlPAG did not alter baseline MAP or HR, and had no effects on the cardiovascular variables in response to a second mechanical stimulation (Fig. 2). Finally, at 120 min after discontinuation of the drug, a third mechanical stimulation also produced no effects on the cardiovascular responses (Table 1). In 5 rats, glutamate and GABA concentrations within the dlPAG as determined by the high-performance liquid chromatography with electrochemical detection (HPLC-