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Research Report

Midbrain modulation of the cardiac baroreflex involves excitation of lateral parabrachial neurons in the rat

*Linda F. Hayward**

University of Florida, College of Veterinary Medicine, Department of Physiological Sciences and McKnight Brain Institute, PO 100144 Gainesville, FL 32610-1044, USA

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ABSTRACT

Activation of the dorsal periaqueductal gray (PAG) evokes defense-like behavior including a marked increase in sympathetic drive and resetting of baroreflex function. The goal of this study was to investigate the role of the lateral parabrachial nucleus (LPBN) in mediating dorsal PAG modulation of the arterial baroreflex. Reflex responses were elicited by electrical stimulation of the aortic depressor nerve (ADN) at 5 Hz or 15 Hz in urethane anesthetized rats ($n=18$). Electrical stimulation of the dorsal PAG at 10 Hz did not alter baseline mean arterial pressure (MAP) but did significantly attenuate baroreflex control of heart rate (HR) evoked by low frequency ADN stimulation. Alternatively, 40 Hz dorsal PAG stimulation increased baseline MAP (43±3 mm Hg) and HR (33±3 bpm) and attenuated baroreflex control of HR at both ADN stimulation frequencies. Reflex control of MAP was generally unchanged by dorsal PAG stimulation. Bilateral inhibition of neurons in LPBN area ($n=6$) with muscimol (0.45 nmol per side) reduced dorsal PAG-evoked increases in MAP and HR by 50±4% and 95±4%, respectively, and significantly reduced, but did not completely eliminate dorsal PAG attenuation of the cardiac baroreflex. Bilateral blockade of glutamate receptors in the LPBN area ($n=6$) with kynurenic acid (1.8 nmol) had a similar effect on dorsal PAG-evoked increases in MAP, HR and cardiac baroreflex function. Reflex control of MAP was unchanged with either treatment. These findings suggest that the LPBN area is one of several brainstem regions involved in descending modulation of the cardiac baroreflex function during defensive behavior.

1. Introduction

In both cat and rat, activation of the dorsal cell column of the periaqueductal gray (PAG) elicits a behavioral response similar to that evoked by threatening or stressful stimuli, including flight or fight responses (Bandler and Shipley, 1994; Bandler and Carrive, 1988; Bandler et al., 1985; Carrive and Karli, 1986; Graeff, 1994; Hunsperger, 1963; Krieger and Graeff, 1985). Coupled with these behavioral changes are coordinated cardiorespiratory responses, including sympathoexcitation, tachycardia, hyperventilation and modulation of baroreflex function (Comet et al., 2004; Hayward et al., 2003; Nosaka et al., 1993; Schenberg et al., 1993; Verberne and Guyenet, 1992). Direct neuroanatomical projections from the dorsal PAG to select sympathoexcitatory regions of the medulla, including the paragigantocellular nucleus (PGi) and rostral ventrolateral medulla (RVLM), have been identified (Cameron et al., 1995; Chen and Aston-Jones, 1995; Farkas et al., 1998; Hermann et

* Fax: +1 352 392 5145.
E-mail address: lindah@ufl.edu.

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al., 1997; Odeh and Antal, 2001; Van Bockstaele et al., 1991), suggesting that a portion of the cardiovascular response evoked by dorsal PAG activation is mediated through a direct pathway to presympathetic bulbospinal neurons (Hilton and Smith, 1984; Li and Lovick, 1985; Lovick, 1992, 1985; Verberne and Guyenet, 1992).

Although direct projections to the RVLM have been identified, current evidence suggests that dorsal PAG modulation of sympathoexcitation also involves an important relay through the rostral pons. In 1993 Nosaka and colleagues first demonstrated that large electrolytic or chemical lesions of the dorsal pons, localized to the region of the parabrachial nucleus (PBN), markedly reduced dorsal PAG-evoked increases in mean arterial pressure (MAP) (Nosaka et al., 1993). In a more recent study from our laboratory, utilizing smaller microinjection volumes, we confirmed that activation of lateral PBN (LPBN) neurons was essential for dorsal PAG-evoked increases in MAP and heart rate (HR), as well as changes in breath timing (Hayward et al., 2004). This observation corroborated neuroanatomical data showing that dorsal PAG neurons primarily project to the LPBN and not the medial PBN (Krout et al., 1998). Unexpectedly however, we demonstrated that bilateral blockade of LPBN area neurons with the GABA-A receptor agonist muscimol or the glutamate receptor antagonist kynurenic acid was approximately 20% more effective at attenuating dorsal PAG-evoked increases in HR than changes in MAP. This raised the possibility that dorsal PAG modulation of HR is primarily mediated through input to the LPBN, while descending control of sympathetic drive to the vasculature involves an alternative pathway to the RVLM.

In addition to inducing increases in sympathetic drive, activation of the dorsal PAG also modulates baroreflex function. Previous studies have shown that PAG stimulation raises both the threshold for baroreflex-mediated inhibition of lumbar and splanchnic sympathetic nerve activity and increases the cut-off pressure needed to silence sympathetic drive (Verberne and Guyenet, 1992). Additionally, activation of dorsal PAG neurons has been reported to attenuate baroreflex control of HR (Comet et al., 2004; Nosaka et al., 1993).

Accordingly, in their original study, Nosaka and colleagues demonstrated that large chemical lesions of the PBN also markedly attenuated dorsal PAG modulation of baroreflex control of MAP and HR (Nosaka et al., 1993). This provided further evidence for a critical role for PBN area neurons in mediating a portion of dorsal PAG modulation of autonomic function. However, because the lesions utilized in that study were relatively large, it remains to be determined whether dorsal PAG modulation of baroreflex function is primarily mediated through neurons in the LPBN or in surrounding regions. Current evidence suggests that dorsolateral pontine-evoked modulation of the arterial baroreflex primarily originates from the ventrolateral regions of the LPBN and involves descending projections to both the NTS (Felder and Mifflin, 1988; Len and Chan, 2001) and the RVLM (Len and Chan, 2001; Len et al., 2000; Len and Chan, 1999; Mraovitch et al., 1982). Yet, neuroanatomical data suggest that there are few direct projections from the dorsal PAG to the ventrolateral PBN (Krout et al., 1998). Instead, descending projections from the dorsal PAG primarily terminate in the central and superior lateral regions of the LPBN. Thus it remains to be determined whether blockade of LPBN neurons using smaller, more centrally focused injections is involved in mediating dorsal PAG modulation of baroreflex function.

Based on the above information, the present study was undertaken to test the hypothesis that the integrity of LPBN area neurons is essential for dorsal PAG modulation of baroreflex function. Additionally, we hypothesized that dorsal PAG modulation of baroreflex control is dependent upon glutamatergic input to the LPBN.

2. Results

The mean weight of the adult male Sprague–Dawley rats used in this study was 375±9 g (n=18). The average resting MAP and HR of all rats prior to central microinjection was 94±3 mm Hg and 384±7 bpm, respectively. Between treatment groups there was no significant difference in weight (data not shown), baseline MAP or HR (see Table 1, $p>0.5$).

2.1. *Effect of dorsal PAG stimulation on baroreflex function*

Fig. 1 illustrates the effect of dorsal PAG stimulation on baroreflex function from one animal. The averaged responses from all animals (n=18) are shown in Fig. 2. Alone, electrical stimulation of the ADN evoked bradycardia and hypotension (Fig. 1A). Reflex decreases in MAP and HR evoked by 5 Hz ADN stimulation (Fig. 2A, open bars) were significantly ($p<0.005$) smaller than those evoked by 15 Hz (Fig. 2B, open bars). Activation of the dorsal PAG at 10 Hz did not significantly increase resting MAP (+2±0.5 mm Hg) or HR (+1±0.5 mm Hg). In contrast, PAG stimulation at 40 Hz induced a ramp increase in baseline MAP and HR that reached a peak approximately 10 s following the onset of PAG stimulation (Fig. 1C) and was significantly different from resting levels (MAP +43±3 mm Hg; HR +33±3 mm Hg). Co-activation of the dorsal PAG modified baroreflex control of HR to a greater extent than reflex control of MAP and 40 Hz PAG stimulation had a greater effect on reflex control than 10 Hz. Reflex control of MAP was only significantly attenuated when 40 Hz PAG stimulation was applied during low frequency–5 Hz ADN stimulation.

One minute following the offset of dorsal PAG stimulation at 40 Hz, resting MAP quickly returned to pre-stimulation levels, but baseline HR remained elevated for several minutes (Fig. 1D). In nine animals baroreflex function (15 Hz ADN stimulation) was retested 1 min following the offset of 40 Hz dorsal PAG stimulation. In these animals dorsal PAG stimulation (40 Hz) alone induced an increase MAP and HR of 49±4 mm Hg and 35±4 bpm above baseline, respectively.

Table 1 – Baseline mean arterial pressure (MAP) and heart rate (HR) before and following bilateral microinjection into the LPBN for each treatment group of rats

	MAP (mm Hg) Pre vs. Post-drug	HR (bpm) Pre vs. Post-drug
aCSF (n=6)	96±3 vs. 96±5	388±15 vs. 401±17
Muscimol (n=6)	97±7 vs. 107±8	383±5 vs. 396±13
Kynurenic acid (n=6)	90±6 vs. 97±6	380±15 vs. 385±17