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Impact of the vagal feedback on cardiorespiratory coupling in anesthetized rats

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ABSTRACT

Cardiorespiratory coupling can be significantly influenced by both pontine and vagal modulation of medullary motor and premotor areas. We investigated influences of the pontine intertrigeminal region (ITR) and peripheral vagal pathways on the coupling between systolic blood pressure (SBP) and respiration in 9 anesthetized rats. Glutamate injection into the ITR perturbed both respiration and SBP and decreased SBP-respiratory coherence (0.95 ± 0.01 vs 0.89 ± 0.02; ($p = 0.01$). Intravenous infusion of serotonin (5-HT) produced apnea and hypertension and also decreased SBP-respiratory coherence (0.95 ± 0.01 vs 0.72 ± 0.06; $p = 0.04$). Bilateral vagotomy eliminated the cardiorespiratory coherence perturbations induced by central (glutamate injection into the ITR: 0.89 ± 0.03 vs 0.86 ± 0.03; $p = 0.63$) and peripheral (5-HT infusion: 0.89 ± 0.03 vs 0.88 ± 0.02; $p = 0.98$) pharmacologic manipulations. Glutamate stimulation of the ITR postvagotomy increased the relative spectral power density of SBP in the respiratory frequency range (0.25 ± 0.08 vs 0.55 ± 0.06; $p = 0.01$). The data suggest that SBP-respiratory coupling is largely mediated within the central nervous system, with vagal systems acting in a way that disrupts coherence during transient cardiorespiratory disturbances. Although decreased cardiorespiratory coherence may increase cardiac work during perturbations, this may be physiologically advantageous in restoring homeostatic equilibrium of respiration and blood pressure.

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1. Introduction

Cardiorespiratory coupling can be regulated by both central and peripheral mechanisms. Pontine structures were shown to play an essential role in coordination of respiratory and cardiovascular activity. It was recently demonstrated that respiratory sinus arrhythmia, respiratory modulation of sympathetic nerve activity (Traube-Hering waves in arterial pressure) and post-inspiratory discharges from vagal efferents were eliminated after pontine transection (Baekey et al., 2008). Pontine nuclei modulate cardiorespiratory afferentation and its primary integration (including modulation of baroreflexes) in the nucleus of the solitary tract (NTS) (Felder and Mifflin, 1988; Mifflin and Felder, 1990; Paton et al., 1990). The pons also may be directly involved in the regulation of cardiorespiratory efferent patterns, generated by structures of ventrolateral medulla (VLM) (Nunez-Abades et al., 1990; Morrison et al., 1994).

The activity of central cardiorespiratory regulating systems is largely dependent on peripheral inputs, including vagal pathways (Kalia and Mesulam, 1980; Kalia and Sullivan, 1982; Dick et al., 2009). Vagal afferents arising from numerous types of receptors influence both respiratory pattern and vascular tone and impact on cardiorespiratory coupling expressed as respiratory sinus arrhythmia, which is suppressed after vagotomy (Eckberg, 1983; Eckberg and Sleight, 1992). Vagotomy has further been suggested to disrupt central coordination of cardiovascular and respiratory activities in the NTS (Loewy and Spyer, 1990; Paton, 1998) and within the efferent circuits of VLM (Feldman and Ellenberger, 1988; Mandel and Schreihofer, 2006), including vagal efferents within the nucleus ambiguus (McAllen and Spyer, 1975, 1976; Neff et al., 2003).

Recent findings indicate that the pontine intertrigeminal region (ITR) – the group of cells in the lateral pons among the fibers between the motor and principal sensory trigeminal nuclei – is involved both in respiratory regulation (Chamberlin and Saper, 1998, 2003; Radulovacki et al., 2003a,b) and cardiovascular control (Topchiy et al., 2009). Anatomical connections of the ITR with

Abbreviations: ITR, intertrigeminal region; NTS, nucleus of the solitary tract; VLM, ventrolateral medulla; RVLM, rostral ventrolateral medulla; ECG, electrocardiogram; TT, total breath duration; CVTT, coefficient of variation of total breath duration; BP, blood pressure; SBP, systolic blood pressure; CVSBP, coefficient of variation of systolic blood pressure; 5-HT, serotonin.

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the nucleus of the solitary tract and the VLM (Chamberlin and Saper, 1992, 1998, 2003; Verner et al., 2008) suggest its integrating role in a wide range of respiratory and cardiovascular pathways (Chamberlin and Saper, 1998). We previously showed that bilateral vagotomy amplified and unmasked increases of systolic blood pressure as well as pulse pressure, induced by ITR stimulation (Topchiy et al., 2009).

In view of the above-mentioned studies, the aim of this work was to explore the impact of ITR perturbation on cardio-respiratory coupling and the role of vagal pathways in these effects. We assessed cardio-respiratory coupling before and after bilateral supranodose vagotomy with and without stimulation of the ITR by glutamate.

2. Experimental procedure

Experiments were performed on 9 spontaneously breathing, anesthetized, adult, male Sprague–Dawley rats (280–300 g, Harlan, Indianapolis, IN). All procedures complied with the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

2.1. Surgical preparation

Rats were anesthetized with a combination of 80 mg/kg ketamine (Abbott Laboratories, North Chicago, IL) and 5 mg/kg xylazine (Phoenix Scientific Inc., St. Joseph, MO) given by intraperitoneal injection. After achieving a stable plane of anesthesia, which was controlled by the absence of a toe-pinch reflex a midline incision was made from anterior on the mandible caudally to the manubrium (from the sternal margin of the neck to the larynx) and the skin was pulled laterally with retractors. The trachea was cannulated. The left sternohyoid and omohyoid muscles and salivary glands were gently retracted by round-tip forceps exposing the external carotid artery and jugular vein. Blunt dissection of perivascular connective tissue was performed. The vagus nerve was identified as the white strand that lies between the common carotid artery and the internal jugular vein. The bifurcation of the carotid artery into the external and internal branches was taken as a landmark.

Rectus capitus and longus coli were divided transversely, using blunt dissection as far rostrally in the field as possible. The part of the vagus nerve in the proximity of the bifurcation of the common carotid artery was traced to the point where it exits the posterior lacerated foramen of the occipital bone of the scull and forms the nodose ganglion. The nodose ganglion was gently isolated by a small round-tip forceps and a silk ligature was threaded under the supranodose vagus nerve. The same procedure was performed at the right side. The ligatures were tightened to achieve vagotomy at the appropriate point in the protocol.

For blood pressure monitoring a catheter was inserted into the left femoral artery and secured by a suture. Another catheter was inserted into the left femoral vein for bolus infusions of 5-HT (5-hydroxytryptamine hydrochloride, MP Biomedical LLC, Aurora, OH).

After the surgical preparation, rats were placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL). The incisor bar was set at the same level as the ear bars (interaural zero).A unilateral osteotomy was made to allow access to rostral lateral pons, and the dura was carefully removed. Two-barrel micropipettes were made using standard filament glass (1 mm × 0.25 mm, A-M Systems, Carlsberg, WA) and a vertical puller (model no. 50-239, Harvard Apparatus Ltd., Kent, England) to obtain an overall tip diameter of 10–20 μm. The micropipette was introduced into the brain on a dorso-ventral axis to allow pressure microinjections into the ITR (AP = −9.30 from bregma; ML = 2.4 from the midline suture; DV = −8.0 from the brain surface; Paxinos and Watson, 2004). The surface of the brain was used as the zero point for the dorso-ventral stereotaxic coordinates.

A millipulse pressure injector (model Picospritzer II, General Valve Co., Fairfield, NJ) was used to inject glutamate (L-glutamic acid monosodium salt, 10 mM, 30 nl, ICN Biomedicals, Aurora, OH) or oil red O-dye (Sigma, St. Louis, MO; solution of 7 mg in 1 ml ethanol) into the ITR. The dose of glutamate was chosen according the previous works (Chamberlin and Saper, 1992, 1998; Radulovacki et al., 2003a,b, 2007), which showed the effectiveness of this amount to evoke the prominent apneic reactions from the ITR. All drugs were dissolved in 0.2 M PBS. Injection volumes were determined by measuring the displacement of the pipette fluid meniscus with a calibrated eyepiece reticle in a binocular stereozoom microscope (model 48920-10, Cole-Parmer, Vernon Hills, IL). In all cases, the target injection volume was 30 nl. According Nicholson (1985) it was suggested that within the first 30 s the effective diffusion radius of this volume of glutamate is limited to approximately 170–180 μm.

2.2. Recording procedure

In each experimental protocol we performed a four-channel recording: (1) arterial blood pressure registered using a Transpac IV transducer (Hospira, Lake Forest, IL); (2) electrocardiogram (ECG) acquired from needle electrodes placed in the left axillary and right flank regions; (3) respiration (Velcro^R Tab-Infant-Ped; Sleepmate^R Technologies); (4) injection marker (logic level pulse provided by the pressure injector). The respiration recording system comprised a 1 cm^2 piezoelectric crystal attached to an elastic band that was fixed around the animal at a substernal level. The crystal provided quantitative measurements of respiratory timing and relative uncalibrated measurements of respiratory volume.

After conventional amplification and filtering (1–50 Hz bandpass; Grass Model 12, West Warwick), the analog data were digitized (sampling frequency 200/s) and recorded using Brain Wave for Windows software (Datawave Systems, Longmont, CO).

2.3. Experimental protocol

Each recording began with a 10 min registration of the baseline activity prior to any injections. In order to identify an ITR apneic site, the pipette was positioned into the stereotaxically defined intertrigeminal area and advanced ventrally in 100 μm increments until glutamate injection produced an apnea at least 2.5 s in duration, corresponding to a ≥50% increase in breath duration, on average.

In all animals at least two repeated glutamate injections were made at the "respiratory effective" site to better document the duration and reproducibility of the response. The injections were separated by a 10 min interval, which was sufficient for the visually assessed respiratory pattern to return to baseline. After the next 10-min interval a bolus of 5-HT (0.05 M, 0.5 μl over 5 s) was infused intravenously, using a Hamilton syringe and infusion pump (model KDS210, KD Scientific Inc., Hollister, MA). Apnea following the 5-HT infusion indicated the integrity of the nodose ganglia and vagal afferent system. Ten minutes later vagotomy was produced by tightening the ligatures placed around the vagus nerves. After a 30-min interval, sufficient for visually evident stabilization of the respiratory pattern, another bolus of 5-HT was infused to confirm the vagal transection rostral to the nodose ganglia. To determine the effect of vagotomy on glutamate stimulation of the ITR, two additional glutamate injections were made at 10-min intervals. Finally, oil red O-dye was microinjected into the functionally identified ITR apneic site to aid in histological verification.