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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.

Keywords

Intertrigeminal region; Vagotomy; Respiration; Blood pressure; Coherence

Disclosure Statement

The authors: Irina Topchiy, Jonathan Waxman, Miodrag Radulovacki and David W Carley do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three (3) years of beginning the work submitted that could inappropriately influence this work.

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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 et al., 1980; 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 and b) and cardiovascular control (Topchiy et al., 2009). Anatomical connections of the ITR with 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 skull 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, 1994; 1998; Radulovacki et al., 2003a and 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 cm² 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 band-pass; 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 μ m increments until glutamate injection produced an apnea at least 2.5 s in duration, corresponding to a $\geq 50\%$ 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 μ l 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.

2.4. Data analysis

An automated adaptive threshold algorithm was used to detect and quantify: the duration (TT) and tidal volume (VT) of each breath, as well as systolic pressure (SBP), diastolic pressure (DBP) and pulse interval for each beat in the blood pressure waveform. Analysis focused on multiple 240-s intervals from each experiment: control, pre-vagotomy, post-vagotomy, and immediately after each glutamate injection or serotonin infusion. Beat by beat values were then linearly interpolated to produce evenly sampled waveforms (10 samples per s) suitable for coherence analysis. A zero-phase 64-point FIR anti-aliasing filter was then applied followed by a downsampling operation.

Coherence represents a linear measure of coupling between two processes, measured here as physiological signals. First, the time series of each signal is Fourier-transformed, creating a frequency domain representation. Then, cross-spectral analysis allows a quantification of linear coupling between the signals. In general, coherence can be determined for every individual frequency or, more commonly, coherence may be assessed over a relevant pre-determined frequency range. Here, we examined coherence specific to the range of respiratory frequencies observed over the entire study. Additionally, we computed the spectral power density of the SBP signal in the respiratory frequency range relative to the total average SBP power (relative spectral power density). Relative spectral power thus represented magnitude of SBP modulation by respiration.

The relative spectral power density and the coherence between SBP and respiration were calculated as follows. The Fourier power spectral density was computed and recorded for each systolic blood pressure and respiratory signal divided into non-overlapping 30 s

segments using a 256 sample Hanning window. A linear detrending operation was applied to each segment prior to computing power spectral densities, and the resulting density values were normalized to the sampling frequency. Coherence between systolic blood pressure and respiration and the relative spectral power density of SBP were computed for each interval within the respiratory frequency range. This range was separately determined for control, pre-vagotomy, post-vagotomy, and post-glutamate injection intervals using the respiratory power spectral densities.

Finally, the eight 30-s segments of each 240 s interval were pooled to produce average coherence and relative power density measurements. This has the advantage of increasing the statistical reliability of the estimates. The interval length of 240 s was chosen in view of our previous data, which showed that respiratory and BP disturbances following infusion of 5-HT or injection of the glutamate into the ITR needs at least 200 s to stabilize.

The mean $\pm$ SE values of all parameters, the relative spectral power density of SBP within the respiratory frequency range and the coherence between SBP and respiration were calculated over 240 s intervals at baseline, just prior to and 30 min after vagotomy, and immediately following each injection (glutamate) or infusion (serotonin).

The effects of glutamate injection, serotonin infusion and vagotomy were assessed by paired t-tests. In all cases, statistical significance was inferred for $p < 0.05$.

2.5. Histology

At the end of each experiment rats were deeply anesthetized and perfused intracardially. The perfusion started with a vascular rinse with 0.9% saline until the liver was cleared (at a perfusion speed of 40 ml/min). The perfusion continued with 4% paraformaldehyde solution (300 ml at 40 ml/min, then 30 ml/min), and finally with 10% sucrose solution in phosphate buffer (30 ml/min). The brain was extracted en-bloc, cleared of meninges and blood vessels, and immersed in 4% paraformaldehyde overnight, and then stored in 30% sucrose (in 0.1 M PBS). Frozen brain tissue was cut in 40 μ m thick sections in a transverse plane using a cryostat microtome (model Leica CM1900, Nussloch, Germany). To identify the location of microinjection sites the sections were stained using cresyl violet Nissl stain.

3. Results

3.1. Effects of vagotomy on cardiorespiratory responses to glutamate injection and serotonin infusion

Table 1 describes the mean $\pm$ SE values for breath by breath and beat by beat parameters of respiration and blood pressure before and after vagotomy. As expected, following vagotomy, TT, VT, heart rate and SBP all increased, although the change in SBP did not achieve statistical significance (Table 1). Fig. 1 depicts typical responses to glutamate injection and serotonin infusion before and after supra-nodose vagotomy. Microstimulation of the ITR by glutamate evoked immediate apnea of 3–10 s in duration, both before (Fig. 1A) and after (Fig. 1B) vagotomy. Transient hypertension appeared simultaneously with the apnea followed by brief hypotension within the first 30 s following glutamate injection (Fig. 1A). Similar transient hypertension was observed after vagotomy, but ensuing hypotension was unusual (Fig. 1B).

The number of apneas following glutamate injection did not differ in pre-vagotomy vs. post-vagotomy trials ($p = 0.1$; Fisher Exact Test), nor did the average duration of evoked apnea (8.82 ± 1.15 vs 9.16 ± 1.35 ; $p = 0.84$; Table 2). The duration of the initial hypertension evoked by glutamate also was unchanged by vagotomy (38.6 ± 5.82 vs 31.73 ± 5.27 ; $p = 0.42$; Table 2).

These responses are typical for glutamatergic stimulation of the ITR as we have previously described (Topchiy et al., 2009).

Fig. 1C and Fig. 1D demonstrate the effects of serotonin infusion before and after supranodose vagotomy. Concomitant apnea and hypertension were observed before vagotomy (Fig. 1C). Following vagotomy, serotonin infusion evoked brief hypertension, with or without ensuing transient hypotension, and apnea was blocked (Fig. 1D). In two cases post-vagotomy infusion of the serotonin evoked apneas, however, these were much shorter than the paired apneas prior to vagotomy (Table 2).

3.2. Effects of Vagotomy on Cardiorespiratory Coupling

Fig. 2 shows the coherence between respiration and SBP after glutamate injections into the ITR and after intravenous serotonin infusions. Prior to vagotomy, ITR stimulation (PreVagX_Glu) significantly reduced the coupling between respiration and SBP (Ctrl: 0.95 ± 0.01 vs PreVagX_Glu: 0.89 ± 0.02 , $p=0.01$). Intravenous infusion of 5-HT also reduced cardiorespiratory coupling prior to vagotomy (Ctrl: 0.95 ± 0.01 vs PreVagX_5HT: 0.72 ± 0.06 , $p=0.04$).

Coherence immediately prior (240 s) to vagotomy (Base_PreVagX) did not differ significantly from the control level (Ctrl: 0.95 ± 0.01 vs Base_PreVagX: 0.95 ± 0.02 ; $p=0.71$), demonstrating the stability of the experimental preparation. Vagotomy *per se* produced a slight decrease in cardiorespiratory coupling (Base_PreVagX vs Base_PostVagX: 0.95 ± 0.02 vs 0.89 ± 0.03 ; $p=0.03$) (Fig. 2).

In contrast with pre-vagotomy conditions, post-vagotomy injection of glutamate into the ITR did not change cardiorespiratory coherence (Base_PostVagX vs PostVagX_Glu: 0.89 ± 0.03 vs 0.86 ± 0.03 ; $p=0.63$). Infusion of serotonin following vagotomy also had no significant effect on cardiorespiratory coupling (Base_PostVagX vs PostVagX_5HT: 0.89 ± 0.03 vs 0.88 ± 0.02 ; $p=0.98$).

Fig. 3 reveals that the relative spectral power density of SBP fluctuations in the respiratory frequency range did not change following injection of glutamate into the ITR (Ctrl: 0.26 ± 0.06 vs PreVagX_Glu: 0.27 ± 0.04 , $p=0.91$). Administration of serotonin also did not produce significant changes (Ctrl: 0.26 ± 0.06 vs PreVagX_5HT: 0.09 ± 0.04 , $p=0.21$) in respiratory frequency power in SBP. Vagotomy *per se* did not alter relative spectral power density (Base_PreVagX vs Base_PostVagX: 0.29 ± 0.07 vs 0.25 ± 0.08 ; $p=0.7$). However, glutamate injection following vagotomy initiated a significant increase of the relative spectral power density of BP in the respiratory range (Base_PostVagX vs PostVagX_Glu: 0.25 ± 0.08 vs 0.55 ± 0.06 ; $p=0.01$).

3.3. Histological localization of injection sites

Histological analysis of the injection sites confirmed the location of the functionally identified apneic points within anatomical boundaries of the ITR (9.0–9.72 mm posterior to bregma) between the principal sensory and motor trigeminal nuclei.

Fig. 4A provides a photomicrographic illustration of an ITR injection site with the micropipette trace. A schematic map of the ITR injection sites is presented in Fig. 4B.

In summary, stimulation of the ITR by glutamate perturbed both respiratory and cardiovascular patterns and decreased coherence between SBP and respiration. Infusion of serotonin also disturbed cardiorespiratory homeostasis by decreasing coupling (decrease of SBP-respiratory coherence). Supranodose vagotomy altered respiratory and blood pressure patterns, and produced a slight, but statistically significant, disruption in cardiorespiratory

coupling. In contrast to pre-vagotomy conditions, glutamate stimulation of the ITR following vagotomy did not change cardiorespiratory coherence, and in fact increased the relative spectral power density of the BP signal in the respiratory frequency range.

4. Discussion

The present study demonstrated that under anesthetized conditions, pharmacologic transient perturbation of respiration and blood pressure by either central (ITR) or peripheral (5-HT) manipulations is associated with reduced coupling between these systems. In addition, we demonstrated a significant impact of vagal pathways on cardiorespiratory coupling. Although bilateral vagotomy slightly reduced the coherence between respiration and SBP, it also eliminated the larger reductions of cardiorespiratory coherence in the face of perturbations induced by pharmacologic manipulations of either the central or peripheral nervous system. Moreover, vagotomy actually increased the magnitude of respiratory modulation in SBP following ITR glutamate injections, which is reflected in the increased spectral power density of SBP in the respiratory frequency range.

The modulatory influences of pontine structures on respiratory pattern and rhythm generation, heart rate, blood pressure and cardiorespiratory coupling are well recognized (Cohen, 1971; von Euler, 1977; Oku and Dick, 1992; Paton et al., 1998; Dick and Coles, 2000; Alheid et al., 2004; Dutschmann and Herbert, 2006; Smith et al., 2007; Baekey et al., 2008; Padley et al., 2008; Segers et al., 1985; 2008). It was shown that pontine transection eliminates respiratory sinus arrhythmia, respiratory modulation of BP (Traube-Hering waves) and postinspiratory discharges from vagal efferents (Baekey et al., 2008). Thus, central pontine influences represent key modulators of cardiorespiratory coupling, including coherent fluctuations in respiration and blood pressure as examined in the present study.

The influence of the pontine ITR on cardiorespiratory outputs has been previously demonstrated (Topchiy et al., 2009). Respiratory and blood pressure perturbations following ITR microinjection with glutamate may be a direct result of activating efferent projections to the respiratory and cardiovascular sites of the ventrolateral medulla (VLM) (Chamberlin and Saper, 1992; 1998; Chamberlin, 2004). The precise synaptic targets of ITR projections are not known, but most probably include respiratory and cardiovascular integrating and premotor neurons of the VLM. Integrated activity of the VLM respiratory and cardiovascular neurons (Miura and Reis, 1972; Koepchen, 1981; Connelly and Wurster, 1985; Loewy and Spyer, 1990) may influence respiratory pattern and motor outputs via the phrenic nucleus and cardiovascular outputs via descending branches of the vagus (Cohen, 1976; Gilbey, 1984; Bianchi et al., 1982; 1995; Pilowsky, 1995; Alheid et al., 2004). ITR influences also may be mediated via VLM projections to the medi thoracic spinal cord area which in turn sends terminals to the heart and vasculature, which are an important component of vasomotor regulation. Further, ITR projects to the medullary raphe (Bernard, 1998; Verberne et al., 1999; Verner et al., 2008), a region interconnected with multiple respiratory and cardiovascular regulatory structures (Lindsey et al., 1998) as well as with other pontine areas (Kölliker-Fuse, parabrachial area).

Because the ITR is functionally embedded within the brainstem homeostatic networks for respiration and blood pressure, it is not surprising that glutamatergic stimulation of the ITR evokes a prominent cardiorespiratory disturbance. The transient decrease of cardiorespiratory coupling after glutamate stimulation of the ITR could be explained by independent activation of respiratory and cardiovascular pathways that interferes with the ongoing coupling of these two systems. The notion of independently activated pathways is consistent with our observation that the blood pressure reaction to ITR stimulation typically outlasted the apneic response.

Assuming SBP-respiratory coherence depended strictly on central pathways, vagotomy would not be expected to significantly alter the impact of ITR stimulation on coherence. The fact that vagotomy blocked the decrease in coherence without reducing the magnitude of the disturbance itself instead argues that the primary source of SBP-respiratory coherence is central, but that vagal feedback processes participating in the integrative response to ITR stimulation operate in a manner that reduces cardiorespiratory coherence.

It was previously shown (Topchiy et al., 2009), that in addition to the immediate effects, stimulation of the ITR by glutamate evoked a delayed increase of SBP. This delayed response is consistent with a possible activation of vasomotor sympathetic outputs following ITR stimulation; responses that build more slowly than do vagal outputs. Thus, the delayed increase of BP could be caused by projections from the ITR to the pressor region of the VLM and subsequently activation of sympathetic premotor neurons, driving vasomotor activity (Preiss et al., 1975; Ross et al., 1984; Reis et al., 1984). The timing of the SBP response fits with the time constant for a sympathetically mediated pressor response. For example, the average time required for the sympathetic pressor response to a cold stimulus is approximately 15 s, whereas the average time for the chronotropic (cardiac vagal) parasympathetic response is only about 5 s (Khurana, 2007). The delayed SBP response is prolonged following vagotomy, suggesting the importance of baroreceptor feedback from the aortic arch receptors in limiting the hypertension. It is also possible that delayed increases in sympathetic activity could be driven by post-apneic hypoxia in addition to or instead of by ITR projections to the VLM. In either case, the net effect of the vagus nerves on responses to ITR stimulation appears to be homeostatic in limiting the blood pressure transient responses, but disruptive to SBP-respiratory coherence.

The negative impact of the vagus nerve on SBP-respiratory coupling is further supported by the observation that serotonin infusion increased blood pressure and reduced coherence to a much greater extent than did ITR stimulation. Further, the decrease of the SBP-respiratory coherence following the serotonin infusion was eliminated by vagotomy, despite the fact that the blood pressure response was intact. Stimulation of both vagal afferent nerve endings and cell bodies within the nodose ganglia can be produced by intravascular infusion of serotonin (Yoshioka et al., 1992a and b). Since intravenous serotonin does not penetrate the blood-brain barrier, the nodose ganglia are considered to be a primary target of its action. This view is supported by the observation that the apneic response to serotonin was virtually abolished after bilateral supranodose vagotomy in the present and previous studies (Jacobs and Comroe, 1971; Segers and Shannon, 1985; Yoshioka et al., 1992a and b; Kopczynska and Szereda-Przestaszewska, 2003).

Intravenous infusion of serotonin (prior to vagotomy) produced prolonged apnea and prominent fluctuation of blood pressure (in most cases hypertension), which either followed or occurred simultaneously with the apnea. A similar effect was described in dogs by Schneider and Yonkman (1954). Prior to vagotomy, infusion of serotonin evoked a large decrease of SBP-respiratory coherence. Following vagotomy, despite the persistence of a large blood pressure disturbance, serotonin infusion did not alter cardiorespiratory coupling. Again, it suggests that vagal activity during transient homeostatic disturbances acts to reduce cardiorespiratory coherence.

Although not the primary source of the apneic response, the hypertension evoked by infusion of 5-HT may have exerted some impact on the overall respiratory response via baroreflex mechanisms. Anatomically, information from baroreceptors is transferred both via the glossopharyngeal nerve (from the carotid sinus) and via the vagus nerve (from the aortic arch). However, in the rat the latter is the main source of both baroreceptor and chemoreceptor afferentation. Supranodose vagotomy removes the cardiac/aortic reflex

pathway, which is carried via the vagus nerve (Krieger and Marsellian, 1963; Faber et al., 1983) – and may therefore have significantly reduced any baroreflex mediated contribution to the apnea.

However, examination of the timing of hypertension and apnea evoked by the infusion of 5-HT revealed that many (but not all) apneas preceded observable hypertension and others appeared simultaneously with blood pressure changes. This fact argues against hypertension as the primary cause of apnea but does not rule out a contribution of baroreflex activation on the apneic response. In addition, pharmacologic hypertension evoked, for example, by infusion of phenylephrine, can prolong expiratory time, but does not commonly evoke apnea. Further, activation of pulmonary vagal stretch receptors provokes apnea via the Hering-Breuer reflex (Younes et al., 1974; Carley et al., 2004) without activation of baroreceptors and 5-HT can elicit dose-dependent apnea via activation of vagal afferents (Yoshioka et al., 1992, 1996; Radulovacki et al., 2007). Together, these facts suggest that the 5-HT evoked apneic response reflects activation of non-baroreceptor vagal afferents.

We thus conclude that respiration and blood pressure are strongly coherent under baseline conditions in anesthetized rats and that this coherence is reduced in the face of pharmacologically induced disturbance in intact anesthetized animals. Conversely, as it was shown in the present study, bilateral vagotomy exerts only minor effects on cardiorespiratory coherence in equilibrium conditions, but eliminates the loss of coherence induced by both stimulation of the pontine ITR and of peripheral serotonin receptors.

In support of our data, considerable experimental evidence indicates that most peripheral and central inputs affect both cardiovascular and respiratory integration and motor outputs, rather than either system alone, allowing speculation as to the physiological importance of cardiorespiratory coupling. It has been suggested for example, that cardiorespiratory coupling may optimize the performance of the cardiac pump by matching cardiac filling to venous return, and that this may be especially important during sleep or anesthesia (Galletly and Larsen, 1997). Our observation of high respiratory-SBP coherence at baseline in anesthetized rats is consistent with this view. However, in the face of transient perturbations to cardiorespiratory equilibrium, it may be physiologically advantageous for the cardiovascular and respiratory control systems to act more independently, as observed in the present experiments. Such independent action may, for example, shorten the time required for return to equilibrium. In particular, in the context of prolonged expiration, as induced by ITR glutamate injection or serotonin infusion in this study, it may be deleterious to maintain strong coupling between respiratory pattern generation and cardiac vagal outputs, as this may produce profound bradycardia (Spyer, 1991). This also is in keeping with the observation of decreased cardiorespiratory coupling in sleep related breathing disorders such as sleep apnea syndrome (Kabir et al., 2010).

Taken together, our observations suggest that coupling between respiration and blood pressure is largely mediated within the central nervous system, with the vagal system acting in a way that disrupts coherence during transient cardiorespiratory disturbances, but that may be physiologically advantageous in restoring homeostatic equilibrium of respiration and blood pressure.

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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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Fig. 1.

Examples of respiratory and blood pressure reactions evoked by glutamate injections into the ITR before the vagotomy (A. PreVagX_Glu), after the vagotomy (B. PostVagX_Glu) and after intravenous infusion of serotonin in pre-vagotomy (C. PreVagX_5HT) and post-vagotomy (D. PostVagX_5HT) periods.

Fig. 2.

Group averages of coherence between systolic blood pressure and respiration calculated over 240 s intervals. Control (Ctrl) vs: baseline records before vagotomy (Base_PreVagX) and after vagotomy (Base_Post_VagX). Control vs: injection of glutamate before vagotomy (PreVagX_Glu) and infusion of serotonin before vagotomy (PreVagX_5HT). Base_Post_VagX vs: intravenous infusion of serotonin post-vagotomy (Post_VagX_5HT) and microinjection of glutamate post-vagotomy (PostVagX_Glu). Results are group means $\pm$ S.E. *Comparison to Ctrl, $p \leq 0.05$. † Comparison to Base_PreVagX, $p \leq 0.05$.

Fig. 3.

Group averages of relative spectral power density of SBP in the respiratory frequency range calculated over 240 s intervals. Control (Ctrl) vs: baseline records before vagotomy (Base_PreVagX) and after vagotomy (Base_PostVagX). Control vs: injection of glutamate before vagotomy (PreVagX_Glu) and infusion of serotonin before vagotomy (PreVagX_5HT). Base_PostVagX vs: intravenous infusion of serotonin post-vagotomy (PostVagX_5HT) and microinjection of glutamate post-vagotomy (PostVagX_Glu). Results are group means $\pm$ S.E. † Comparison to Base_PostVagX, $p \leq 0.05$.

A **B**

Fig. 4.
A. Histologic verification of the ITR apneic site. ITR: intertrigeminal region; Mo5: motor trigeminal nucleus; Pr5: principal sensory trigeminal nucleus. B. Schematic diagram of the histologically verified injections into apneic ITR sites. The arrow indicates the injection site for the recordings, shown in Fig. 1.

Table 1

The mean total breath time (TT), tidal volume (VT) , systolic blood pressure (SBP) and heart rate (HR) in pre-vagotomy (PreVagX) and post- vagotomy (PostVagX) conditions

	TT (s)		VT (A.U.)		SBP (mm Hg)		HR(beat/min)	
	PreVagX	PostVagX	PreVagX	PostVagX	PreVagX	PostVagX	PreVagX	PostVagX
	1.66±0.17	2.23±0.21	88.28±13	145.86±13.8	158.94±10.03	169.16±9.98	195.9±13.39	217.5±20.03
p-values	0.002		0.03		0.43		0.04	

Table 1 shows group means ± standard errors,
* p ≤ 0.05 with respect to pre-injection of glutamate. A.U. – arbitrary units.

Table 2

Duration of apnea and BP reaction in pre-vagotomy and post-vagotomy conditions, following injection of glutamate into the ITR and intravenous infusion of serotonin

	Apnea length (s)			BP reaction (s)		
	Glu		5-HT	Glu		5-HT
	PreVag_X	PostVag_X	PreVag_X	PostVag_X	PreVag_X	PostVag_X
AVG	8.82±1.15	9.16±1.35	21.54±7.46	4.18±0.82	38.6±5.82	31.73±5.27
N	9	9	6	2	9	6
P value	0.84		0.06		0.42	
					0.03	

P-values were considered significant if ≤ 0.05 .