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The effect of tracheal occlusion on respiratory load compensation: Changes in neurons containing inhibitory neurotransmitter in the nucleus of the solitary tract in conscious rats☆

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ABSTRACT

Respiratory load compensation volume–time (V_t–T) relationships have been extensively studied in anesthetized animals. There are only a few studies in conscious animals although consciousness and behavior play a critical role in modulation of breathing. The aims of the study were to determine the effect of intermittent and transient tracheal occlusions (ITTO) elicited load compensation responses and the changes in activation of inhibitory glycinergic neurons in the nucleus of solitary tract (NTS) in conscious rats. The results showed that ITTO elicited an increase in expiratory time (T_e) but did not affect inspiratory time (T_i) and diaphragm activity (EMG_{dia}). An increase in total breathing time (T_{tot}) was due exclusively to the increase in T_e. In addition, glycinergic neurons were activated in the intermediate NTS (iNTS) but not in the caudal NTS (cNTS). These results suggest that the activated glycinergic neurons in the iNTS may be important for the neurogenesis of load compensation responses in conscious animals.

1. Introduction

Respiratory load compensation has been extensively studied in anesthetized animals. There are relatively few studies of load compensation in the conscious state although consciousness and behavior play a critical role in the modulation of breathing pattern when animals encounter breathing challenges.

Respiratory load compensation responses are characterized as volume–time (V_t–T) relationships in anesthetized animals. A respiratory load is a mechanical opposition to ventilation such as airway resistance, lung compliance or thoracic mechanical impedance. Applying a single inspiratory or expiratory load decreased volume of inspired (V_i) and expired air (V_e) and resulted in a prolongation of inspiratory time (T_i) and expiratory time (T_e), respectively; the timing parameters were not affected during unloaded breaths (Clark and von Euler, 1972; Zechman et al., 1976). This is a vagal-dependent reflex and mediated primarily by slowly adapting pulmonary stretch receptors (PSRs) in lungs and airways. PSRs respond to changes in lung volume or transmural pressure across the airways and synapse with second order interneurons in the nucleus of solitary tract (NTS) that project to the pontine respiratory group (PRG) and ventral respiratory group (VRG) to modify breathing pattern (Davenport et al., 1981a,b; Davenport and Wozniak, 1986). In addition to the V_t–T relationships, respiratory load compensation is also characterized by an increase of diaphragm activity and an recruitment of abdominal muscle motor output (Koehler and Bishop, 1979; Lopata et al., 1983). In anesthetized animals, the load compensation responses are principally mediated by the neurons in the brainstem respiratory neural network (Pate and Davenport, 2012; Tsai and Davenport, 2014).

In conscious animals, respiratory load compensation involves cortical (primary sensorimotor cortices, supplementary motor and premotor cortex) and subcortical neural structures (thalamus, globus pallidus, caudate, cerebellum and limbic system) to voluntarily modify breathing pattern (Davenport and Vovk, 2009). It has been demonstrated that there is a gating system in the thalamus for the perception of respiratory load sensation in humans (Chan and Davenport, 2008). In addition, neurons in the thalamus were activated by tracheal occlusions in rats which may be related to the gating process of respiratory load sensation (Pate and Davenport, 2013). A gated in signal will be processed and integrated by the limbic system (affective dimension) and somatosensory cortex (discriminative dimension) (von Leupoldt and Dahme, 2005, 2007). The affective dimension is responsible for the processing of

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the emotional component of the respiratory inputs (Davenport and Vovk, 2009). The discriminative dimension is related to the determination of the spatial, temporal and intensity perception of the respiratory inputs (von Leupoldt and Dahme, 2005; von Leupoldt et al., 2008). The motor cortex receives the integrated sensory information and sends projections to the brainstem or directly to the motor neurons in the spinal cord to allow voluntarily modification of the reflexive pattern of load compensation (Davenport and Vovk, 2009).

Conscious animals respond to respiratory load differently from anesthetized animals. In conscious dogs, single tracheal occlusion at the end of expiration prolonged T_i and decreased breathing frequency (Phillipson, 1974). Conscious newborn lambs responded to a single expiratory load by dilating the larynx and prolonging T_e, followed by a return to baseline during the post-load breath (Watts et al., 1997). Application of two consecutive external inspiratory loads to conscious goats caused prolonged T_i and augmented respiratory output during the two loaded breaths (Hutt et al., 1991). In awake ponies, sustained external inspiratory loads for 4 min resulted in an increase in T_i, decrease in T_e and augmentation of diaphragm activity during the first loaded breath and only had minimal changes after first loaded breath (Forster et al., 1994). In conscious humans, application of resistive loads for an entire breath resulted in a prolongation of T_i, T_e and total breathing time (T_{tot}) (Calabrese et al., 1998). The conflicting results between these studies may be due to different experimental designs, including differences in load strengths, load durations, load applications, species and age. Previous studies used external respiratory loads to elicit load compensation responses and applied the load to a single phase of respiration (inspiration or expiration) or a single entire breath. To our knowledge, respiratory load compensation elicited by transient and intrinsic tracheal occlusions in conscious animals has not been studied. In our laboratory, we developed a surgical strategy to produce intrinsic, transient tracheal occlusions (ITTO) without changing lung compliance. The first purpose of the present study was to determine the ITTO-elicited load compensation responses in conscious animals.

Additionally, respiratory drive in anesthetized animal is dominated by the reciprocal interconnections within the brainstem neural network. In the model proposed by Rybak et al. (2007, 2008), the brainstem respiratory neural network consists of synaptic connections between the pons, ventral medulla and nucleus of solitary tract. Inhibitory reciprocal interconnections between the post-I, aug-E in the BötC and pre-I, early-I in the pre-BötC are proposed to generate the respiratory rhythm. The pons has excitatory projections to the respiratory neurons in the dorsal and ventral medulla to modulate the rhythm and pattern of breathing. The NTS is a site of integrating and processing respiratory peripheral afferents from lungs and airways for further modification of breathing pattern to generate appropriate ventilation for the body's demand. PSRs in the lungs and airways are the primary receptors sensing the changes in lung volume or transmural pressure and synapse on the pump cells in the NTS and project to other respiratory neurons in the medulla and pons for the reflex control of breathing pattern (Davenport et al., 1981a). In addition, It has been reported that application of excitatory amino acids on the medial subnucleus of solitary tract (SolM) containing pump cells caused reflex termination of inspiration and prolongation of expiration, while blockade of excitatory amino acid in this area reduced these changes (Bonham et al., 1993; Bonham and McCrimmon, 1990).

It has been demonstrated that glycinergic neurons in the NTS were activated by tracheal occlusions in anesthetized animals (Tsai and Davenport, 2014). Therefore, the inhibitory glycinergic neurons in the NTS are critically important for the neurogenesis of load compensation responses. In conscious animals, the breathing pattern is principally modulated by cortical and subcortical structures implying that consciousness and behavior are important for the neurogenesis of conscious load compensation responses. Therefore, the second purpose of this study was to determine the distribution and activation of inhibitory glycinergic neurons in the NTS responding to ITTO in conscious animals. We hypothesized that glycinergic neurons in the NTS would be activated by ITTO in conscious animals. We used immunofluorescence double staining of c-Fos and glycine transporter 2 (GlyT2) to test our hypothesis.

2. Materials and methods

2.1. Animals

Experiments were performed on 11 male Sprague-Dawley rats (320–380 g). The animals were housed in the University of Florida animal care facility. They were exposed to a 12 h light/12 h dark cycle and with free access to food and water. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Florida.

2.2. Surgical procedure

The Sprague- Dawley rats were randomly divided into experimental ($n=5$) and sham ($n=6$) groups. All animals were anesthetized using inhaled isoflurane gas (2–5% in O_2) and received a tracheal cuff and bipolar electrodes in diaphragm. Adequate anesthetic depth was verified by the absence of a withdrawal reflex to a rear paw pinch. Buprenorphine (0.04–0.05 mg/kg body weight) and carprofen (5 mg/kg body weight) were administered preoperatively via subcutaneous injection. The eyes were coated with petroleum ointment to prevent drying. Incision sites were shaved and sterilized with povidine–iodine topical antiseptic solution. Body temperature was monitored with a rectal probe and maintained at 37–39 °C using a heating pad.

The trachea was exposed through a ventral incision. The trachea was separated from surrounding tissue. An inflatable cuff (Fine Science Tools; Foster City, CA, USA) was sutured around the trachea, two cartilage rings caudal to the larynx. The cuff was connected to a saline-filled syringe via a rubber tube. An exploratory laparotomy was performed and bipolar electrodes were implanted into the diaphragm. The rubber tube from the cuff and electrodes were routed subcutaneously to the dorsal neck surface and externalized through an incision between the scapulae, and were fixed in the skin using closing sutures. The ventral neck incision and abdominal incision were closed using an interrupted suture pattern.

Following surgery, rats were administered normal saline (0.01–0.02 ml/g body weight). Postoperative analgesia was provided with buprenorphine (0.01–0.05 mg/kg body weight) and carprofen (5 mg/kg body weight) every 24 h for at least three days. Animals were allowed a full week recovery before training protocol began.

2.3. Experimental protocol

One week after the surgery, the rats were placed in a restrainer for 3 h per day for two days for habituation. On post-operative day10, experimental group rats were placed in a restrainer for 90 min, followed by 10 min of ITTO then 90 min post-occlusion eupneic breathing in the restrainer; sham group rats were placed in a restrainer for 3 h without receiving ITTO. ITTO was initiated in the beginning of inspiration and prolonged for 2–3 s. During the experiment, diaphragm EMG (EMG_{dia}) were recorded by bipolar electrodes implanted in diaphragm and the signal was fed into an amplifier (P511, Grass Instruments; Quincy, MA) and band-pass filtered (30–3000 Hz). The analog output was digitized at 5 kHz, processed and stored for analysis. The trachea cuff was connected