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Learning to breathe: Habituation of Hering–Breuer inflation reflex emerges with postnatal brainstem maturation

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ABSTRACT

The Hering–Breuer (HBR) reflex is considered a major regulatory feedback for the generation and patterning of respiratory activity. While HBR is important in neonates, its significance in adults is controversial. Previous experiments that investigated the plasticity of entrainment of the respiratory rhythm by vagal input demonstrated postnatal changes in HBR plasticity. Here we analyzed postnatal changes in the plasticity of HBR by mimicking the classic lung inflation tests with repetitive tonic vagal stimulation across different postnatal stages in an in situ perfused brainstem preparation of rat. The study shows that neonates stereotypically exhibit HBR stimulus-dependent prolongation of expiration while juvenile preparations (>postnatal day 16) showed significant habituation of HBR following repetitive stimulation. Subsequent experiments employing physiological lung inflation tests in situ confirmed HBR habituation in juveniles. We conclude that postnatal emergence of HBR habituation explains the weak contribution and high activation threshold of HBR in the regulation of eupnea.

1. Introduction

The Hering–Breuer inflation reflex (HBR, Hering, 1868; Breuer, 1868) is a 'classic' reflex in respiratory control that is described in practically every physiology textbook. In brief, lung inflation activates slowly adapting pulmonary stretch receptors. The receptor input is relayed via the vagus nerve to 'pump' cells located in and around the ventro-lateral nucleus of the solitary tract (Clark and Euler, 1972; Berger and Dick, 1987; Bonham and McCrimmon, 1990; Bonham et al., 1993; Miyazaki et al., 1998, 1999). Pump cells project to and release inhibitory neurotransmitters on inspiratory neurons in the lateral respiratory column (Ezure and Tanaka, 2004; see Kubin et al., 2006); thus, contributing to the termination of inspiration (see Euler, 1981, 1983; Bianchi et al., 1995; Kubin et al., 2006).

The HBR is often considered as an inhibitory sensory feedback loop that shapes the respiratory motor pattern. Removing HBR feedback via cooling of the vagal nerve or vagotomy instantly transforms the respiratory pattern to a slower breathing rhythm with increased inspiratory amplitude and longer duration of expiration (Marckwald, 1887; Stella, 1938, Clark and Euler, 1972).

Although the HBR is an established mechanism, doubts about its physiological significance arose from findings that show little or no effect of the HBR in adult humans (summarized in Kubin et al., 2006). In particular, the HBR does not affect the respiratory pattern in humans until a high lung volume is reached. Thus, the threshold for HBR is well above end-inspiratory lung volume during resting breathing (Bechbache et al., 1979; Cunningham and Gardner, 1977; Duffin et al., 2000). In contrast, the HBR is important for the stabilization of the breathing pattern in neonatal rats (Fedorko et al., 1988). So, the HBR appears to becomes less significant with postnatal maturation of the cardio-respiratory control circuits as observed in humans (Gerhardt and Bancalari, 1981; Rabbette et al., 1991; Rabbette and Stocks, 1998; BuSha et al., 2002) and mammals in general (Trippenbach, 1994).

A plausible explanation for diminished role of the HBR in adults is provided by pioneering investigations of Chi-Sang Poon and co-workers demonstrating that the HBR habituates in rats (Siniaia et al., 2000; Poon, 2004; Song and Poon, 2004; MacDonald et al., 2009; Tadjalli et al., 2010). Thus, if postnatal maturation of brainstem circuitry is mandatory before these networks become permissive for synaptic plasticity (see Dutschmann et al., 2004, 2008, 2009; Dutschmann and Dick, 2012), then HBR habituation does not occur in the neonatal stages of brainstem development.

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Therefore we investigated HBR habituation in juvenile rat by using repetitive brief electrical stimulation of the central vagus nerve across 3 different stages of postnatal brainstem development. Habituation is the decrease in strength of the response to a stimulus that is presented repeatedly. In the present study, the initial vagal stimuli suppressed inspiratory activity but as the stimulus was repeated, the phrenic bursting 'broke-through' during vagal stimulation. The phrenic bursting during the stimulus indicated habituation. Importantly, HBR habituation did not emerge until postnatal day 16. Subsequent experiments show an identical habituation of inspiratory suppression in response to repetitively applied sustained lung inflation in juvenile rat preparations. The experiments using physiological stimulus of sustained lung inflation are consistent with a postnatal emergence of synaptic plasticity linked to HBR habituation.

2. Materials and methods

Experiments were performed at the Georg August University Göttingen (Germany) and at the Florey Institute of Neuroscience and Mental Health (Australia). Approval for experiments was obtained from both Institutional Animal Care Ethics Committees. The experimental procedures were performed in accordance with international guidelines for the care and use of laboratory animals.

2.1. Perfused brainstem preparations

The arterially perfused brainstem preparation of rat at various postnatal stages was used, as previously described in detail (Dutschmann et al., 2009). Here phrenic nerve activity (PNA) was recorded as an index of the respiratory motor output. Respiratory motor output was optimized by adjusting the flow rate (5–22 ml min^{-1}) and perfusion pressure (40–70 mmHg) depended on the age of the rat pup used for the preparation.

2.2. Electrical stimulation of the vagus and lung inflation tests

For vagus nerve stimulation, we used the stimulus parameters of our previous publication (Dutschmann et al., 2009). In brief, the effect of fictive feedback from pulmonary stretch receptors at different postnatal stages was simulated by electrical stimulation of central branch of the vagus nerve (Master 8, A.M.P.I., pulse duration: 50–100 μs; stimulus frequency: 20 Hz, stimulus duration: 10 s, stimulus intensity: 0.2–1 mA). The vagus nerve was stimulated 1.5× the stimulus threshold that terminated inspiration at end inspiration (Stanley et al., 1975). After threshold determination, we applied repetitive vagal stimulation: 15 consecutive trials separated by a 2 min interval.

We conducted these experiments in preparations of different postnatal ages which were divided into three age groups: (A) a neonatal group (postnatal day (P4–8), (B) an intermediate group (P9–15) and (C) a juvenile group (P16–21).

For sustained lung inflation tests in juvenile rat preparations, the right lung lobes were left intact during the initial surgery. The trachea was intubated and connected to a small rodent ventilator (SAR 830/P, CWE Inc., USA). Initially, we used rhythmic lung ventilation at a frequency of 30 breaths/min and increased ventilation pressure (mmH_2O) until individual lung inflations terminated PNA bursts. After determining the threshold, the inflation pressure was increased by 50% (60–85 mmH_2O) and 15 lung inflations of 10 s duration were delivered at 2-min intervals.

2.3. Data analysis

For both vagal stimulation and sustained lung inflation protocols, PNA was used to analyze the following respiratory variables: total respiratory cycle length (T_{TOT}), durations of inspiration (T_I) and expiration (T_E). These variables were analyzed 1 min before, during and after the 10 s stimulations/inflations. Progressive changes in T_E prolongation evoked by either vagal stimulation or lung inflation were tested for significance using regression analyses Analysis of Covariance (ANCOVA, Systat). ANCOVA was also used for the analysis of progressive changes in respiratory variables before and after vagal stimulation and lung inflations. The post-stimulus rebound (desensitization) was analyzed by calculating the time required until T_{TOT} returned to baseline values determined from the pre-stimulus period. Changes in respiratory activity before (baseline) and after the vagal stimulation protocol were analyzed using a two-tailed, paired *t*-test.

3. Results

3.1. Developmental changes in inspiratory depression in response to Hering–Breuer inflation reflex (HBR)

Postnatal maturation of HBR is illustrated by representative examples of the response to repetitive tonic vagal nerve simulation in a neonatal (Fig. 1A) and juvenile preparation (Fig. 1B). In each age group, the first stimulus consistently suppressed PNA for 10 s at least (see Fig. 1). In preparations of the neonatal age group ($n = 5$), analysis of vagally mediated T_E prolongation was not significantly different between the 1st (11.82 ± 0.41 s) and 15th (12.26 ± 0.46 s) stimulus (see Fig. 2 ANCOVA, n.s.). A similar time course of vagally evoked T_E prolongation was observed in the intermediate age group (1st stimulation: 11.96 ± 0.87 s vs. 15th stimulation: 11.37 ± 0.24 s; $n = 5$ preparations; Fig. 2 ANCOVA, n.s.). However, in the juvenile age group ($n = 5$), the repetitive vagal stimulation displayed habituation of the fictive HBR response because PNA bursts emerged during the acute vagal stimulation (see Fig. 1B). Consequently, vagally evoked T_E prolongation became progressively shorter (1st stimulation: 12.1 ± 0.40 s; 15th stimulation: 6.69 ± 0.83 s, Fig. 2; ANCOVA, $p < 0.001$).

3.2. Post-stimulus rebound

In each experimental group, post-stimulus rebound activity was characterized by a transient increases in respiratory frequency (f_R) compared to pre-stimulus f_R after termination of vagal stimulation (indicated as decrease in T_{TOT} illustrated in Fig. 1C). The time required for the f_R returned to pre-stimulus values remained unchanged in the neonatal age group as shown in Fig. 2B (4.6 ± 0.7 s, 1st stimulation vs 6.7 ± 1.7 s, 15th stimulation). In the intermediate age group the rebound period was substantially longer compared to neonates (*t*-test, $p < 0.05$). However, repetitive vagal stimulus trials did not change the duration of the rebound significantly (12.6 ± 3 s, 1st trial vs. 14.8 ±1.4 s, 15th trail). Similar to habituation, only the juvenile age group showed plasticity of the post-stimulus rebound indicated by a significant prolongation of the rebound period (13.4 ± 3.9 s, 1st trial vs. 21 ± 5.8 s, 15th trail, Fig. 2B; ANCOVA $p < 0.05$).

3.3. Changes in baseline respiration

In neonates, the vagal stimulation protocol had no significant effect on timing of the respiratory phases after the trial; comparing the breathing pattern before (baseline) to that after the stimulation protocol (T_{TOT}: 1.92 ± 0.04 s vs. 1.66 ± 0.26 s, T_I: 0.36 ± 0.02 s vs. 0.32 ± 0.02 s, T_E 1.56 ± 0.04 s vs. 1.34 ± 0.24 s). However, in the intermediate and juvenile groups, consecutive vagal stimulation periods caused a subtle but significant increase in respiratory drive. In the intermediate group, T_{TOT} decreased from 1.92 ± 0.10 s to 1.61 ± 0.13 s, ($p = 0.05$) and T_I, from 0.49 ± 0.05 s to 0.42 ± 0.04 s,