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Respiratory modulation of sympathetic nerve activity is enhanced in male rat offspring following uteroplacental insufficiency

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ABSTRACT

Sympathetic nerve activity to the cardiovascular system displays prominent respiratory-related modulation which leads to the generation of rhythmic oscillations in blood pressure called Traube–Hering waves. An amplification of this respiratory modulation of sympathetic activity is observed in hypertension of both genetic, the spontaneously hypertensive rat, and induced, chronic intermittent hypoxia or maternal protein restriction during gestation, origin. Male offspring of mothers with uteroplacental insufficiency, induced by bilateral uterine vessel ligation at 18 days of gestation, are also hypertensive in adulthood. In this study we examined whether these male offspring display altered respiratory modulation of sympathetic activity at pre-hypertensive ages compared to controls. Respiratory, cardiovascular and sympathetic parameters were examined using the working heart–brainstem preparation in 35 day old male rats that had reduced birth weight due to uteroplacental insufficiency. Whilst all respiratory parameters were not different between groups, we observed an enhanced respiratory-related burst of thoracic sympathetic nerve activity and amplified Traube–Hering waves in the growth-restricted group. This group also showed an increased sympathetic and bradycardic response to activation of peripheral chemoreceptors. The observations add support to the view that altered respiratory modulation of sympathetic activity represents a common mechanism involved in the development of several forms of hypertension.

1. Introduction

The aetiology of hypertension remains poorly understood, however, one commonly observed feature is an elevation in the sympathetic nerve activity (Schlaich et al., 2007). Sympathetic nerve activity shows rhythmic oscillations that occur in phase with the respiratory rhythm (Adrian et al., 1932) and there is accumulating evidence that this respiratory modulation of sympathetic activity is altered during the development of hypertension. For example rats exposed to chronic intermittent hypoxia develop hypertension and display an increase in respiratory-related sympathetic drive with an additional burst in phase with late-expiration (Zoccal et al., 2008). In juvenile Spontaneously Hypertensive Rats (SHR), prior to the development of hypertension, respiratory modulation of sympathetic activity is dramatically increased during the inspiratory/post-inspiratory phase (Simms et al., 2009). Similarly, rat pups subjected to maternal protein restriction during gestation and lactation, which show severe growth restriction, also develop an increase in respiratory-related sympathetic activity before the onset of hypertension (De Brito Alves et al., 2015). Together, these studies support the idea that an altered interaction between central respiratory and sympathetic circuits contribute to the development of hypertension.

Bilateral uterine vessel ligation at gestational day 18 induces uteroplacental insufficiency resulting in offspring that have reduced birth weight. The male offspring develop a small, but significant, hypertension in adulthood (Wlodek et al., 2008). A reduction in nephron number has been reported, and may be a contributing factor to the development of hypertension in these growth-restricted offspring (Wlodek et al., 2008). However, the effect of uteroplacental insufficiency on sympathetic activity during development, and prior to the onset of hypertension, has not been examined.

2. Materials and methods

2.1. Animal procedures

All experiments were conducted in accordance with the Australian National Health and Medical Research Council 'Code of

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Practice for the Care and Use of Animals for Scientific Purposes' and were approved by the University of Melbourne Animal Experimentation Ethics and Biosafety Committee. Animals were housed with a 12-h light: dark cycle at a constant temperature (22 ± 1 °C) with ad libitum access to standard rat chow and water. Wistar–Kyoto (WKY) rats (9–13 weeks of age) were mated, and surgery was performed on gestational day 18 (Wlodek et al., 2008). In brief, pregnant rats were randomly allocated to a sham treatment group (offspring termed "control") or uteroplacental insufficiency group (offspring termed "restricted"). The restricted group underwent bilateral uterine vessel (artery and vein) ligation surgery to induce uteroplacental insufficiency. The following experiments were performed on postnatal (PN) day 35 in male offspring (n = 9/group, each from different litters).

2.2. Working heart–brainstem preparation (WHBP)

Recordings of respiratory, sympathetic and cardiovascular activities were made in PN35 restricted (n = 9) and control (n = 9) WKY rats using the WHBP as described previously (Simms et al., 2009). Following reperfusion of the preparations, the final perfusion flow rate was set at 30 ml/min in all animals. Simultaneous recordings of phrenic nerve activity (PNA), thoracic sympathetic nerve activity (tSNA, T8–10), vagus nerve activity (VNA) and abdominal nerve activity (AbNA, T9–T12, recorded in 5 restricted and 4 control rats) were obtained using glass suction electrodes, amplified (10 kHz, Neurolog), filtered (50–1500 kHz, Neurolog), digitized (CED, Cambridge, UK) and recorded to hard disk using Spike2 (CED). Heart rate (HR) was derived by using a window discriminator to trigger from the R-wave of the electrocardiogram recorded simultaneously through the phrenic nerve suction electrode. Peripheral chemoreceptors were stimulated using sodium cyanide (NaCN; 0.05%; 100 μl bolus) injected into the preparation aorta via the perfusion catheter.

2.3. Data analysis and statistics

The signals were rectified and integrated with a 50 ms time constant. The electrical noise levels for tSNA recordings were determined at the end of experiments by sectioning the sympathetic chain at the proximal paravertebral ganglion level, and were subtracted for data analysis. PNA, VNA and AbNA were used to assess respiratory parameters associated with inspiration, pre-inspiration/inspiration/post-inspiration and post-inspiration/late-expiration, respectively. During basal condition, phrenic triggered (end of inspiratory burst) averaging of tSNA (across ≈100 phrenic cycles) was used for the analysis of all tSNA parameters and in particular those related to the burst of respiratory modulation of tSNA. Tonic levels of tSNA were measured during the expiratory period, outside of the burst of respiratory modulation of SNA. Phrenic triggered averaging of systolic PP and HR were used to determine the amplitudes of Traube–Hering waves (mmHg) and respiratory sinus arrhythmia (bpm), respectively. The effect of NaCN application on the different parameters analysed was measured in delta variation between a pre-stimulus control period and the peak NaCN response. Sympathoexcitation and expiratory drive were measured as delta increase in area under the curve of NaCN-induced tSNA and AbNA bursts, respectively, compared to the area under the curve of a control period with same duration. Group data are presented as mean ± s.e.m. Normal distribution of the data was tested using the Shapiro–Wilk test. Normally-distributed data were compared using unpaired Student's t-tests, non-normally distributed data (HR in both groups) were compared

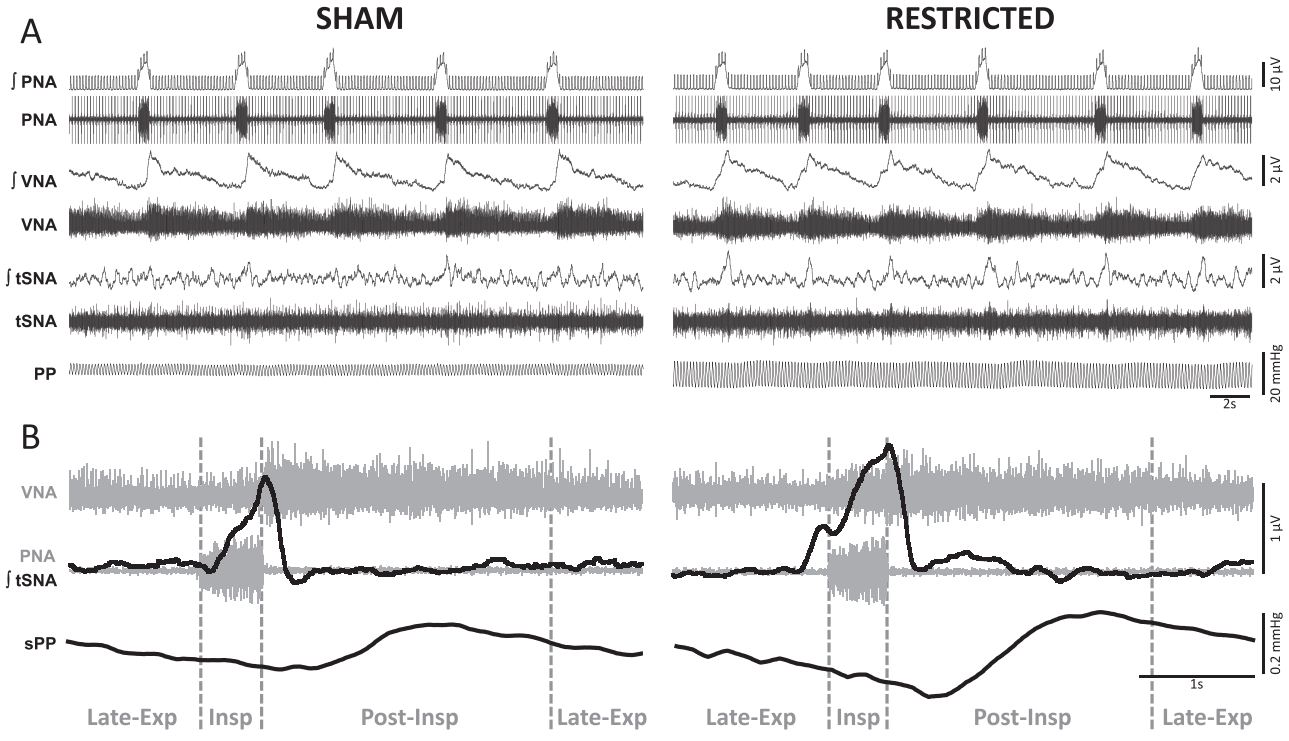

Fig. 1. P35 restricted rats show enlarged respiratory-modulated sympathetic nerve activity and Traube–Hering waves in the WHBP in basal condition compared to control rats. A, raw and integrated traces showing similar respiratory control between control (left panel) and restricted (right panel) rats, but larger bursts of respiratory-modulated tSNA in restricted rats. B, Phrenic triggered averaging of integrated tSNA and sPP (black traces) over raw PNA and VNA (grey traces) showing temporal relationship between these activities in relation to the three phases of the respiratory cycle. Note the larger respiratory-modulated tSNA in restricted (right panel) compared to control (left panel) rat, inducing larger Traube–Hering waves on sPP. ∫, integrated activity; Insp, inspiration; Late-exp, late-expiration; PNA, phrenic nerve activity; Post-insp, post-inspiration; PP, perfusion pressure; sPP, systolic perfusion pressure; tSNA, thoracic sympathetic nerve activity; VNA, vagus nerve activity.