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Respiratory control and sternohyoid muscle structure and function in aged male rats: Decreased susceptibility to chronic intermittent hypoxia

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ABSTRACT

Obstructive sleep apnoea syndrome (OSAS) is a common respiratory disorder characterized by chronic intermittent hypoxia (CIH). We have shown that CIH causes upper airway muscle dysfunction in the rat due to oxidative stress. Ageing is an independent risk factor for the development of OSAS perhaps due to respiratory muscle remodelling and increased susceptibility to hypoxia. We sought to examine the effects of CIH on breathing and pharyngeal dilator muscle structure and function in aged rats. Aged (18–20 months), male Wistar rats were exposed to alternating cycles of normoxia and hypoxia (90 s each; F_IO_2 = 5% O_2 at nadir) or sham treatment for 8 h/day for 9 days. Following CIH exposure, breathing was assessed by whole-body plethysmography. In addition, sternohyoid muscle contractile and endurance properties were examined *in vitro*. Muscle fibre type and cross-sectional area, and the activity of key oxidative and glycolytic enzymes were determined. CIH had no effect on basal breathing or ventilatory responses to hypoxia or hypercapnia. CIH did not alter succinate dehydrogenase or glycerol phosphate dehydrogenase enzyme activities, myosin heavy chain fibre areal density or cross-sectional area. Sternohyoid muscle force and endurance were unaffected by CIH exposure. Since we have established that this CIH paradigm causes sternohyoid muscle weakness in adult male rats, we conclude that aged rats have decreased susceptibility to CIH-induced stress. We suggest that structural remodelling with improved hypoxic tolerance in upper airway muscles may partly compensate for impaired neural regulation of the upper airway and increased propensity for airway collapse in aged mammals.

1. Introduction

Owing to its multifunctional role in breathing, swallowing and speech, the human upper airway is vulnerable to collapse. Obstructive sleep apnoea syndrome (OSAS) is a common (Young et al., 2009), debilitating respiratory disorder associated with significant cardiovascular and neurocognitive morbidities (White, 2005). OSAS is characterized by recurrent collapse of the upper airway impeding pulmonary airflow resulting in episodic bouts of hypoxaemia. The disorder is widely recognized to be a consequence of sleep-related decrements in upper airway muscle activity (Wheatley et al., 1993; Horner et al., 1994; White, 2005) in individuals predisposed to airway occlusion due to abnormal airway anatomy (White, 2005). Respiratory muscle weakness and fatigue are implicated in the pathophysiology of OSAS. There is evidence of upper airway muscle dysfunction in OSAS (Stauffer et al., 1989; Sériès et al., 1996b; Ferini-Strambi et al., 1998; Carrera et al., 1999) and in the English bulldog (Petrof et al., 1994), a naturally occurring animal model of the disorder. In recent years, translational animal models have emerged providing convincing evidence that chronic intermittent hypoxia (CIH) – a hallmark feature of OSAS due to recurrent apnoea – is a key factor in the development of significant morbidities in OSAS patients. For example, CIH in rodents causes systemic (Fletcher, 2000; McGuire and Bradford, 2001) and pulmonary hypertension (McGuire and Bradford, 2001), impaired learning and memory (Gozal et al., 2003; Row et al., 2003), hypersomnolence (Veasey et al., 2004a; Zhan et al., 2005), and insulin resistance (Iiyori et al., 2007; Levy et al., 2008)—morbidities often reported in OSAS patients (White, 2005). Several groups have also demonstrated that CIH alters respiratory control (Ling et al., 2001; O'Halloran et al., 2002; Reeves et al., 2003; Veasey et al., 2004b; Reeves and Gozal, 2006; Ray et al., 2007; Edge et al., 2010; Skelly et al., 2011a), and causes respiratory muscle dysfunction (McGuire

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et al., 2002b; Pae et al., 2005; Farkas et al., 2007; Dunleavy et al., 2008; Skelly et al., 2010b, 2011a) which may have special relevance to OSAS and other respiratory muscle weakness disorders.

Advancing age is an independent risk factor for the development of OSAS (White, 2005). Ageing is associated with structural and functional remodelling of skeletal muscle (Degens and Alway, 2006), including the upper airway muscles (Van Lunteren et al., 1995; Cantillon and Bradford, 2000), and upper airway collapsibility increases with age (Ray et al., 2008). Slow-to-fast fibre transitions (Van Lunteren et al., 1995), decreased oxidative capacity (Oliven et al., 2001) and increased fatigue (Van Lunteren et al., 1995) have been described in aged upper airway dilator muscles. However, despite the clinical relevance, the effects of CIH on pharyngeal dilator muscle function in aged animals are unknown though it has been shown that ageing increases susceptibility to CIH-induced neural injury (Gozal et al., 2003). Therefore, using a CIH paradigm that was recently shown in our laboratory to cause respiratory muscle impairment in young adult male rats (Skelly et al., 2010b, 2011a), we examined the effects of CIH on breathing and pharyngeal dilator muscle structure and function in aged male rats. We chose to study the sternohyoid muscle because it is an important airway dilator muscle (Roberts et al., 1984; Van Lunteren et al., 1987), which shows evidence of remodelling in the English Bulldog (Petrof et al., 1994)—a natural model of OSAS. The muscle is readily accessible and its fibres are arranged longitudinally which makes it ideal for *in vitro* assessment of contractile function. We hypothesized that CIH would cause pronounced respiratory muscle dysfunction in aged animals.

2. Materials and methods

2.1. Ethical approval

All protocols described in this study were performed under licence from the Irish Government Department of Health and Children in accordance with National and European guidelines following institutional ethics committee approval.

2.2. Chronic intermittent hypoxia

Experiments were performed on 13 aged (18–20 months) male Wistar rats (569 ± 72 g, mean ± SD). Rats were placed unrestrained in environmental chambers with free access to food and water. CIH treatment consisted of alternating 90 s cycles of normoxia (21% O_2) and hypoxia (reaching 5% O_2 at the nadir, SaO_2 ~80%; see Fig. 1). Sham rats were exposed to alternating cycles of normoxia under identical experimental conditions in studies that were conducted in parallel. Exposures lasted 8 h/day for 9 treatment days.

2.3. Whole-body plethysmography

On the day after gas treatments were completed, respiratory parameters were measured by whole-body plethysmography in unrestrained rats during quiet rest. Animals were introduced into plethysmograph chambers (Buxco Europe Ltd.) and were allowed a 30–60 min settling period with humidified air passing through the chambers (2 l/min). Ventilatory parameters were logged every 10 breaths and stored for later analysis. An algorithm in the Buxco software analysis program allowed the exclusion of motion-induced artifacts. Measurements were temperature and humidity compensated and corrected for body temperature. During quiet rest, a 10 min baseline period of stable ventilation was recorded in normoxia. This was followed by a 6 min hypercapnic challenge (5% CO_2; balance O_2). Following recovery, a second 10 min normoxic baseline period was recorded. Next, a 6 min hypoxic challenge (10% O_2; balance nitrogen) was performed. A final 10 min normoxic baseline period was recorded.

2.4. Muscle physiology

The paired sternohyoid muscles were separated and placed in a bath at room temperature containing continuously gassed (95% O_2/5% CO_2) physiological solution containing NaCl 120 mM, KCl 5 mM, Ca^{2+} gluconate 2.5 mM, $MgSO_4$ 1.2 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 25 mM, glucose 11.5 mM and D-tubocurarine (25 μM). Longitudinal strips of muscle (1–2 mm in diameter) were placed vertically in one of two water-jacketed organ baths at 35 °C gassed with either 95% O_2/5% CO_2 for control studies or 95% N_2/5% CO_2 to create tissue hypoxia (bath PO_2 ~45 mm Hg). Thus for a given rat, muscle function was assessed in both hyperoxia and hypoxia using separate muscle bundles. We have previously established that sternohyoid force and endurance is optimum under hyperoxic compared to normoxic conditions (Skelly et al., 2010a). Thus bath hyperoxia was chosen as the optimum control condition. The muscle strips were positioned between a pair of platinum plate electrodes, with the base fixed to an immobile hook and the other end tied to an isometric force transducer with non-elastic string. Strips were equilibrated for 5 min. The position of the force transducer could be adjusted by a micropositioner thus altering the length of the muscle strips which was measured *in situ* using a fixed graduated scale.

2.5. Muscle function protocol

The optimum length (L_O, muscle length producing maximal isometric twitch force in response to supra-maximal stimulation) was determined by incrementally adjusting the micropositioner between intermittent stimulations. Once determined, the muscle remained at this length for the full protocol. Next the muscle was allowed a 5 min equilibration period. The single isometric twitch force, contraction time, half-relaxation time, force–frequency relationship and performance during repeated stimulation were then determined in response to electrical field stimulation using a well established experimental protocol (Cantillon and Bradford, 1998, 2000; McGuire et al., 2002a; O'Halloran, 2006; Dunleavy et al., 2008; Skelly et al., 2010a,b, 2011a,b; McMorrow et al., 2011). First, a single twitch was elicited (supra-maximal voltage, 1 ms duration). Twitch force, contraction time (time to peak force) and half-relaxation time (time for peak force to decay by 50%) were determined. Next, force–frequency relationship was determined by sequentially stimulating the muscle strips at 10, 20, 30, 40, 60, 80 and 100 Hz for 300 ms at each stimulus frequency allowing a 2 min recovery interval between each stimulus. Five min following this force–frequency protocol, repeated muscle contraction was induced by stimulation at 40 Hz with 300 ms trains every 2 s for a period of 2 min.

2.6. SDH and GPDH enzyme histochemistry

Longitudinal muscle strips from sham and CIH-treated animals were snap frozen in isopentane, cooled in liquid nitrogen, for approximately 10 s and were subsequently coated in Tissue Tek (OCT mounting medium, VWR, Pennsylvania, USA) before being dipped again in isopentane for a further 20–30 s. Muscles were then stored at −80 °C until later use. Serial transverse 10 μm sections were cryosectioned (Model CM30505, Leica Microsystems, Nussloch, Germany) at −22 °C and were mounted on polysine-coated glass slides. Muscle sections were stained histochemically for the mitochondrial enzyme succinate dehydrogenase (SDH). SDH activity was determined using an incubation solution containing sodium succinate and nitro blue tetrazolium chloride (NBT) in phosphate