Respiratory Physiology & Neurobiology 250 (2018) 31–38

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Hypoglossal nerve stimulation in a pre-clinical anesthetized rabbit model relevant to OSA

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ARTICLE INFO

Keywords:
Rabbit
Hypoglossal nerve stimulation
Obstructive sleep apnea
Upper airway

ABSTRACT

We tested the functional effects of hypoglossal (CNXII) stimulation in the anesthetized rabbit before and after injections of saline into the tongue base to obstruct the airway. Data (n = 6) show little or no effect of CN XII trunk stimulation; however, medial branch stimulation (20–100 Hz; 50–500 μs pulse width, and incremental increases from 10 μA) reduced upper airway resistance. Medial branch stimulation was less effective in reducing resistance than anterior advancement of the hyoid. Endoscopic viewing (n-3) of the retropalate showed this region as the narrowest and dynamically changed by anterior hyoid displacement, with less evident effects than CNXII stimulation. We conclude that under these conditions CNXII medial branch stimulation reduces airway resistance, especially after induced obstruction.

1. Introduction

Obstructive sleep apnea (OSA) is a human disorder characterized by recurrent complete or partial obstruction of the pharyngeal airway at the retropalatal and retrolingual airway subsites during sleep, producing nocturnal hypoxemia, hypercapnia and sleep fragmentation (Pham and Schwartz, 2015). Symptomatic OSA occurs in 3–9% of adult women and 10–17% of adult men (Drager et al., 2015). Untreated, it creates daytime sleepiness and is associated with increased risks of hypertension, metabolic disorders and cardiovascular morbidity and mortality. Although continuous positive airway pressure (CPAP) and mandibular advancement devices can restore upper airway patency during sleep, these may be poorly tolerated and/or only partially effective (Dempsey et al., 2010). The development of stimulation approaches suffers from the lack of appropriate pre-clinical models, which could permit a dissection of pathways for and physiologic testing of stimulation treatments of OSA.

Many animal models focus on the consequences of apnea, using exposure to intermittent hypoxia or to repetitive arousals (Chopra et al., 2016; Davis and O'Donnell, 2013; Drager et al., 2015). Few models address causality. In the case of OSA in the bulldog, the relative stability of the pharyngeal walls (due to immobility of the hyoid bone) during NREM sleep results in events occuring largely during REM sleep (Veasey et al., 2001; Veasey et al., 1999; Veasey et al., 1996). Elephant seals have obstructive apneas in NREM and REM sleep and are also inconvenient experimental models (Castellini et al., 1994; Milsom et al., 1996). Mice and rats exhibit central apneas and pauses when breathing during sleep; however, while inexpensive and genetically malleable, rodents do not generally have obstructive apneas (Davis and O'Donnell, 2013). Also the rodent, along with the dog and cat, have a pharyngeal airway anatomy which is comparatively rigid, as the hyoid bone in these species is firmly attached by cartilage to the styloid process and thyroid cartilage, making the airway less collapsible spontaneously or if challenged with an obstruction (Davis and O'Donnell, 2013). Hence, other development of other preclinical animal models could be useful.

The rabbit could be an appropriate model. It has an upper airway pharyngeal structure that most resembles the human. The similarities in the rabbit anatomy to human pharyngeal anatomy makes it vulnerable to collapse (Yu et al., 2014). The hyoid bone of the rabbit, like that of a human, is anchored by muscular attachments rather than by cartilage or bone. The anatomy and branching of the CNXII in the rabbit has relevant similarities to that of the human (Delaey et al., 2017). In the rabbit, OSA may be induced by Botox injection into the hypoglossal nerve, causing inadequate neural activation, or by creating a small mass at the base of the tongue or in the soft palate, thereby altering the pharyngeal anatomy (Liu et al., 2015; Yu et al., 2014).

Currently, hypoglossal nerve stimulation (HNS: Inspire Medical Systems LLC, Maple Grove MN) is an effective treatment of OSA in

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https://doi.org/10.1016/j.resp.2018.01.015
Received 11 October 2017; Received in revised form 27 January 2018; Accepted 29 January 2018
Available online 02 February 2018
1569-9048/

those patients intolerant to CPAP therapy. It works by activation of the muscles innervated by CNXII to prevent passive collapse and blockage of airway during sleep (Mwenge et al., 2015). The placement of electrodes is on the medial branch of CN XII (Van de Heyning et al., 2012). An animal model can be used to compare positions along the nerve for stimulation effectiveness, for instance the trunk vs. one of its branches, according to flow resistance; and one may observe measures of where the airway opens, especially in comparison to anterior displacement of the hyoid (Van de Graaff et al., 1984).

This report details successes and shortcomings of HNS stimulation vs. displacement of the hyoid on upper airway flow resistance and the opening of the pharyngeal airway, the latter as visualized by an endoscope placed from below, through the trachea (Strohl et al., 1986; Van de Graaff et al., 1984). Evaluations were conducted with and without an occluded pharyngeal airway.

2. Materials and methods

All surgical and experimental procedures were approved by Case Western Reserve University and the Louis Stokes Cleveland VA Medical Center, Institutional animal care and use committee and were carried out in accordance with the NIH and the guide for the care and use of laboratory animals. Three rabbits were used to refine protocols on hyoid depression and the parameters used for hypoglossal never trunk and medical branch stimulation. Complete datasets were available from 6 Male NZW rabbits, with a mean weight of 2.5–4.0 kg. Three rabbits (2 preliminary animals and one complete) were instrumented for video endoscopy observations. We chose male rabbits as female rabbits have a dewlap; humans of either gender do not have this appendage of the anterior pharyngeal wall. All rabbits were purchased from Charles River and kept in humidity and temperature controlled facility with a 12 h light/dark cycle. The rabbits were given free access to food, water and enrichment in the form of fresh vegetables and species approved toys and all housed in individual cages with aspen bedding. Study Design is shown in Fig. 1.

2.1. Animal sedation and preparation

Using sterile techniques, rabbits were pre-medicated with Buprenorphine (0.02 mg/kg) SQ, and anesthetized with Propofol (up to 14 mg/kg) IV through the marginal ear vein, followed by atraumatic intubation and Isoflurane (1–5%) gas was then administered via endotracheal tube. Marcaine (4 mg/kg) was used as an analgesic prior to all incisions. A surgical level of anesthesia is evidenced by: 1) no response to toe pinch, 2) relaxed jaw tone and 3) diminished palpebral reflex. The respiratory rate (RR), heart rate (HR), body temperature, and peripheral capillary oxygen saturation (SpO2) were recorded using Bionet BM3VET Next (Bionet America, Inc. Tustin, CA). Pulse strength and reflex responses are manually collected by technician. For surgical procedures, we placed the animal in the dorsal position on a warm water circulating heating pad to maintain a body temperature of 100.4-104.0° F. Animals were transitioned to urethane (1.5 g/kg) administered via IV following the dissection and nerve cuff placement.

2.2. Hypoglossal nerve exposure

The surgical procedure was performed under binocular magnification. A 3 cm vertical incision was made in the ventral midline. The dissection was directed lateral to the laryngotracheal complex and medial to the vascular sheath. The greater horn of the hyoid was identified and just deep to that structure the main trunk of the hypoglossal nerve could be reliably found (Ates et al., 2011). Using a fine microdissection technique, the nerve was carefully mobilized distally until it separated into medial and lateral branches. The vasa nervorum was left largely intact during this microdissection. The mylohyoid muscle (Fig. 2) was retracted for dissection of the medial branch which was mobilized sufficiently to allow for atraumatic stimulation of the nerve. A 3-0 silk suture was placed around the body of the hyoid in the midline to facilitate hyoid advancement. A 16 gauge polyethylene catheter with multiple side holes is inserted directly through a tracheotomy between the third and fourth tracheal rings, positioned towards the distal trachea. During the recording process it is repositioned to face the subglottic region. The catheter is placed below the glottis and secured where it entered the trachea with a suture to limit air leakage.

2.3. Anatomy and monitoring in the study

A schematic model of the preparation is shown in Fig. 3. The side port on a muzzle-mask securely fit to snout and mouth is referenced to the tracheal pressure to provide measures of upper airway pressure (Validyne Model MC 1–3, Validyne Engineering, Northridge CA). The muzzle-mask is connected to a pneumotachograph to measure bulk airflow (Validyne MP-45, Validyne Engineering, Northridge CA). A bipolar stimulating hook or nerve cuff (Microprobes for Life Science, Gaithersburg, MD) is placed on the trunk or medial branch of hypoglossal nerve. Stimulations were conducted using Natus (formerly Grass Instruments) S48 adjustable stimulator (Natus Neurology, Middleton, WI) and a Natus Photoelectric stimulus isolation unit constant current output model # PSIU6.

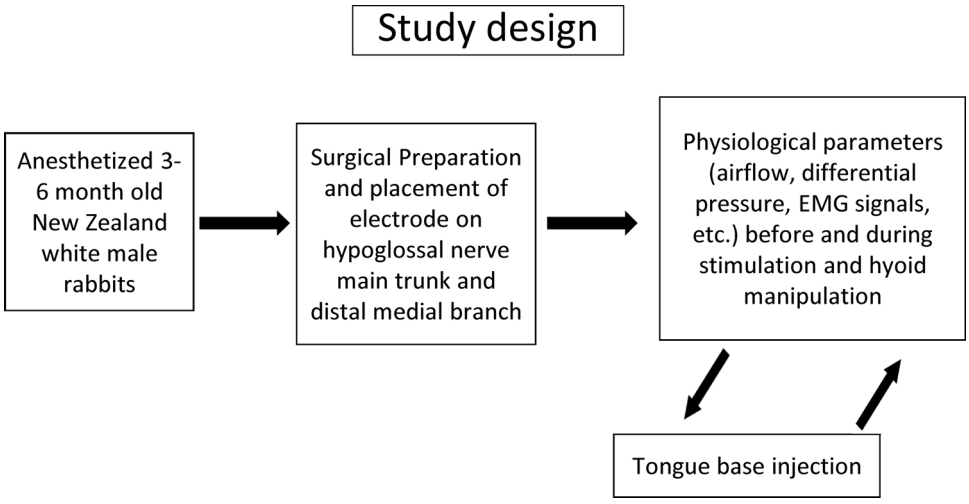

Fig. 1. The experimental sequence of events. Further details are provided in the manuscript.