Editorial

Re-awakening the carotid bodies after anaesthesia: managing hypnotic and neuromuscular blocking agents

M. Raju[1] and **J. J. Pandit**[2]

1 Erasmus Scholar, 2 Consultant, Nuffield Department of Anaesthesia, Oxford University Hospitals NHS Foundation Trust, Oxford, UK

Correspondence to: J. J. Pandit
Email: jaideep.pandit@dpag.ox.ac.uk
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Neuromuscular blocking drugs (NMB) are used in just under half of all general anaesthetics in the UK [1]. However, they have their own unintended effects, especially during early recovery from surgery. We now know that one of these is the risk of accidental awareness during general anaesthesia, which occurs almost exclusively in patients who are paralysed with NMBs [2]. Accidental awareness during recovery arises from too early re-awakening from hypnotic effects of anaesthesia coupled with delayed reversal of paralysis [2]. Postoperative respiratory effects of residual neuromuscular blockade are another well-known side-effects, self-evident if the diaphragm or intercostal muscles are weak or if there is a degree of upper airway collapse [3]. Such clinically detectable paralysis after surgery is at worst evidenced by dyspnoea, agitation, obvious weakness and, if the vocal cords are also partially paralysed, difficulty speaking or coughing (and this last can lead to atelectasis or aspiration). Together these can create a dangerous and frightening situation for patients. Indeed, personal histories from the UK's 5th National Audit Project (NAP5) report suggest that these respiratory symptoms can be interpreted by patients as a perception of having been accidentally awake 'during anaesthesia' [2].

A distinct and different respiratory effect of NMBs is, perhaps, less widely known but is very well established: the specific depression of chemoreflex control of breathing. The peripheral arterial chemoreceptors, located in the carotid bodies, are normally responsible for the rapid response to changing partial pressures of O_2 and CO_2 (hypoxia and hypercapnia, respectively). In humans, the aortic bodies are vestigial, and the central chemoreceptors are not relevant to our discussion because their response time is slow and NMBs, in any case, do not cross the blood brain barrier. In a series of ground-breaking studies in the 1990s, Eriksson's group demonstrated that neuromuscular blockade in volunteers depressed the acute ventilatory response to hypoxia and also to hypercapnia, at subclinical doses that did not depress baseline minute ventilation [4]. This underlines the important distinction in the physiology of the respiratory control system between, on the one hand 'baseline activity' and on the other hand, 'responsiveness'. The former is the innate drive to breathe, representing the 'central rhythm generator' activity of respiratory centre neurones (itself, in part, driven by 'feedforward' mechanisms such as volitional effort). The responsiveness, on the other hand, represents the unconscious 'feedback' part of the control system; the strength of the reflex being measured by its 'gain' or 'sensitivity'. Baseline activity and responsiveness are separate entities and can be influenced differentially. The clinical significance is that a normal resting minute ventilation cannot be taken to imply that the patient can reliably respond appropriately with hyperventilation to a stimulus like hypoxia. Hypoxaemia can arise for several reasons peri-operatively and being unable to respond to it can lead to adverse consequences [5]. This more subtle impairment of chemoreflex function, in addition to clinically evident hypoventilation, may underlie emerging concerns that high dosing of NMBs intra-operatively might adversely influence outcomes [6].

In this issue of *Anaesthesia*, Christensson et al. recruited volunteers with untreated obstructive sleep apnoea (OSA), administered rocuronium (to a train-of-four,

TOF, level ~0.7) and then made them hypoxic and hypercapnic [7]. Neuromuscular blockade was not reversed but allowed to wear off naturally, and respiratory measurements were taken at baseline and at TOF ~0.7 and > 0.9. Their important finding was that, even when TOF 0.7 did not impair baseline ventilation or cause symptomatic muscle weakness, the acute hypoxic ventilatory response was significantly depressed (but in this patient group, the hypercapnic ventilatory response was not). One of the challenges of studying ventilatory control in patients with OSA is that measured expired minute ventilation may be reduced by upper airway collapse and, hence, not reflect the output of the respiratory 'central rhythm generator'. Christensson et al. elegantly overcame this challenge by using background continuous positive airway pressure (CPAP), which they confirmed itself did not influence ventilatory or chemoreflex measurements. In this article, we discuss the implications of these results.

Depression of hypoxic chemoreflex

There are several competing theories of how oxygen is sensed in the carotid body, but one suggested sequence of events is as follows (Fig. 1) [8]. Hypoxia closes background K+ (TASK) channels in the type-1 glomus cells. These leak channels normally serve to maintain membrane potential and hypoxia-induced closure depolarises the glomus cell membrane, which in turn opens voltage-gated Ca^{2+} channels leading to Ca^{2+} influx. The raised intracellular Ca^{2+} (the magnitude of which is proportional to the magnitude of hypoxic stimulus) promotes fusion of neurotransmitter-containing vesicles with the cell membrane. Neurotransmitter exocytosis, and then binding onto specific receptors on the postsynaptic terminal, leads to action potentials in the afferent glossopharyngeal nerve, the frequency of which are proportional to the strength of hypoxic stimulus. The carotid body is rich in neurotransmitters: acetylcholine (ACh), gamma aminobutyric acid (GABA), substance P, dopamine and other catecholamines, adenosine nucleotides, serotonin, enkephalins, neuropeptide Y, calcitonin gene-related peptide, galanin, endothelins and some others are all implicated. Animal studies using an isolated carotid body-nerve preparation have confirmed that non-depolarising neuromuscular blockade inhibits this response to hypoxia [9]. However, nicotinic antagonism does not inhibit the response of the isolated glomus cell to hypoxia [10] and these results together indicate that the action of drugs like rocuronium is at the synapse, not directly on the glomus cell and, in turn, that ACh is a clinically-relevant neurotransmitter at this junction [11].

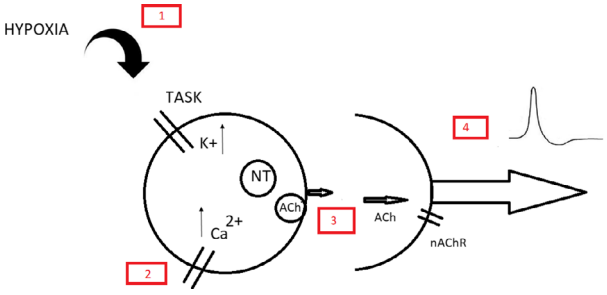

Figure 1 Schematic for oxygen sensing at type-1 glomus cell of carotid body. (1) Hypoxia closes background K+ (TASK) channels, which normally permit background leak of K+ outside the cell; K+ is thus retained in the cell, causing depolarisation. (2) Depolarisation opens voltage-gated Ca^{2+} channels, leading to Ca^{2+} influx. (3) This causes fusion of vesicles containing neurotransmitters (NT) with the cell membrane and acetylcholine (ACh; the likely clinically-relevant neurotransmitter) is released into the synaptic cleft. (4) ACh binds to specific nicotinic receptors (nAChR) causing action potentials in the afferent glossopharyngeal neve, which travel to the respiratory centre. Volatile anaesthetics block the oxygen sensing by TASK channels at step (1). Propofol inhibits glomus cell response by an as yet undefined mechanism (possibly inhibiting voltage-gated Ca^{2+} channels at (2); see reference [14]). Neuromuscular blockade prevents binding of ACh at nAChR at (4).

Christensson et al. report that, when TOF returned to near-normal > 0.9 in their subjects with OSA, acute hypoxic ventilatory response was also restored. However, the effect was variable across individuals and, recently, it has been reported in healthy volunteers, that even near-complete TOF reversal with sugammadex still leaves some individuals with a depressed acute hypoxic ventilatory response (A. Dahan, personal communication). How is it possible that hypoxic response can be depressed by NMBs, even when there is little or no muscle weakness? The human carotid body expresses nicotinic subunits α3, α7 β2 of the pentameric neuronal subtype ACh receptor; skeletal muscle expresses α1, β1, γ, δ or ε subunits. In the latter, up to ~75% of these receptors must be occupied before there is detectable twitch tension reduction. However, in the carotid body, receptors are blocked dose dependently [11]. This difference in receptor structure, and hence in sensitivity to agents, explains why, in some individuals, acute hypoxic ventilatory response is reduced even when neuromuscular function has largely recovered.

However, NMBs are not the only drugs known to depress the peripheral chemoreflex. Very low residual concentrations (<0.1 minimum alveolar concentration) of general anaesthetics (hypnotics) profoundly depress acute hypoxic ventilatory response in humans [12] and this has

been confirmed in human studies [13], animal work [14] and single cell and single channel recordings (cell attached patch clamping of isolated glomus cells) [15, 16]. This research has also established that general anaesthetics prevent the closing of TASK channels by hypoxia with a specific order of potency: halothane > enflurane > isoflurane > sevoflurane, with desflurane being similar in (minimal) depressive potency with sevoflurane [17]. Inhalational anaesthetic agents hence inhibit the whole pathway presented in Fig. 1. Propofol also depresses the isolated glomus cell hypoxic response but by a unique non-TASK, non-nicotinic, non-GABA, non-5-HT mechanism [10]. In other words, the combination of residual general anaesthetics directly depressing the glomus cell by various mechanisms and of NMBs depressing the synapse, is potentially synergistic in profoundly depressing the overall hypoxic response. In clinical practice there may be further depressive synergism with residual concentrations of other agents such as benzodiazepines [18] and opioids [19] which act, not at the carotid body, but more centrally in the nervous system to depress ventilation.

Clinical implications

Clinical outcome studies have found an increased incidence of postoperative desaturation when NMBs are used [20]. If residual neuromuscular blockade is depressive to hypoxic response, then it is logical to reverse it. The choice of reversal agent and timing of administration are, therefore, relevant. There are some theoretical reasons why sugammadex should reverse NMBs more completely than neostigmine. One is that the dose of neostigmine is limited, as excessive dosing can exacerbate paralysis. A second is that even in therapeutic doses some studies report that neostigmine can induce muscle weakness [21]. In contrast, reversal of rocuronium-induced paralysis appears quicker and more complete with sugammadex with fewer adverse outcomes [22].

Monitoring is relevant, as it influences the optimum timing of reversal. Administration of reversal is generally most effective when paralysis is partial as opposed to deep, and depth of paralysis can only be estimated with quantitative neuromuscular function monitoring. Notwithstanding debates around the optimum stimulus type or muscle group used, it is notable that the Association of Anaesthetists' standards of monitoring mandate nerve stimulator monitoring when NMBs are used [23]. There is emerging evidence that quality improvement strategies to record TOF and reverse neuromuscular blockade appropriately can reduce postoperative pulmonary complications [24]. Moreover, the advice is to maintain neuromuscular blockade as light (as many twitches, or as high a TOF) as possible while facilitating surgery or avoid them altogether. This lesson originally emanated from NAP5, where it was realised that traumatic experiences resulted primarily from the awareness of being paralysed, not from the awareness of surgery or even of experiencing pain [2]. Minimising depth of paralysis may allow a patient to signify their awareness. Thus, NMB management is important not only for good restoration of muscle power but also to limit respiratory and, perhaps unexpectedly, psychological side-effects.

The known synergy of NMB-induced depression of hypoxic response with other agents requires us to consider which agents to use in patients at high risk of respiratory complications, like those with OSA. In addition to opioid-sparing techniques, the physiological data make it logical to use agents with less depressive properties to acute hypoxic ventilatory response like sevoflurane, desflurane (or propofol in a total intravenous technique). Advantageously, these are also the agents generally most rapidly eliminated from the body. Additionally, agents like doxapram can be used in clinical practice to stimulate breathing immediately post-surgery. Doxapram is now known to act in a manner akin to hypoxia at the TASK channel of the carotid body glomus cell [25]. However, if postoperative depression of carotid body function arises due to the multimodal action of several drugs themselves acting at multiple sites, then simplistic antagonism at one site (TASK) may not be sufficient, but recent research is focussing on other, specific agents that could stimulate breathing in rational ways [25].

Eikermann has referred to the 'hidden universality of neuromuscular block' [26], by which he had in mind that even patients we believe are fully reversed from NMBs may, in fact, still be partially paralysed. 'Universality' of neuromuscular blockade could apply also in the sense in which NMBs affect not only the musculature but also the respiratory control system. Thus, we need to view complete reversal from neuromuscular blockade as a necessary, but not sufficient end-point for ensuring reversal of hypoxic depression. If there is still detectable paralysis, we can also be sure there will be hypoxic chemoreflex depression.

Many years ago, Knill and Gelb summarized their pioneering work on low-dose anaesthetic effects in inhibiting carotid body function as demonstrating that the 'watchdogs were sleeping' [13]. The very organs tasked to defend us from hypoxaemia remained silenced by residual hypnotics at the time they were, arguably, needed most, in the immediate postoperative period. The careful work of Christensson et al., and others, reminds us that to re-awaken the 'watchdogs', we need to carefully manage

neuromuscular blockade – not just anaesthesia reversal – for a safe recovery.

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Original Article

Hypoxic ventilatory response after rocuronium-induced partial neuromuscular blockade in men with obstructive sleep apnoea*

E. Christensson,[1,2] **A. Ebberyd,**[3] **A. Hårdemark Cedborg,**[4] **Å. Lodenius,**[4] **Å. Österlund Modalen,**[5] **K. A. Franklin,**[6] **L. I. Eriksson**[1,7] and **M. Jonsson Fagerlund**[1,8]

1 Senior Consultant, Function Peri-operative Medicine and Intensive Care, Karolinska University Hospital, Section for Anaesthesiology and Intensive Care Medicine, Karolinska Institute, Stockholm, Sweden
2 PhD-Student, 3 Biomedical Assistant, 4 PhD and Senior Consultant, 7 Professor, 8 Associate Professor, Department of Physiology and Pharmacology, Section for Anaesthesiology and Intensive Care Medicine, Karolinska Institute, Stockholm, Sweden
5 PhD, Department of Sleep Medicine, Aleris fysiologLab, Stockholm, Sweden
6 Senior Lecturer, Department of Surgical and Perioperative Sciences, Surgery, Umeå University, Umeå, Sweden

Summary

Obstructive sleep apnoea and residual neuromuscular blockade are, independently, known to be risk factors for respiratory complications after major surgery. Residual effects of neuromuscular blocking agents are known to reduce the hypoxic ventilatory response in healthy volunteers. Patients with obstructive sleep apnoea have impaired control of breathing, but it is not known to what extent neuromuscular blocking agents interfere with the regulation of breathing in such patients. In a physiological study in 10 unsedated men with untreated obstructive sleep apnoea, we wished to examine if partial neuromuscular blockade had an effect on hypoxic ventilatory response (isocapnic hypoxia to oxygen saturation of 80%) and hypercapnic ventilatory response (normoxic inspired carbon dioxide 5%). The hypoxic ventilatory response was reduced by 32% ($p = 0.016$) during residual neuromuscular block (rocuronium to train-of-four ratio 0.7), but the hypercapnic ventilatory response was unaffected. We conclude that neuromuscular blockade specifically depresses peripheral chemosensitivity, and not respiratory muscle function since the hypercapnic ventilatory response was unaffected.

Correspondence to: E. Christensson
Email: eva.christensson@sll.se
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Twitter: @ChristenssonEva, @FagerlundMalin

Introduction

Residual neuromuscular blockade is associated with pulmonary complications and critical respiratory events in the early postoperative period [1–4]. In healthy volunteers, residual neuromuscular blockade corresponding to a train-of-four (TOF) ratio of 0.70 has been shown to reduce the mean hypoxic ventilatory response (HVR) by one-third [5–7]. This finding is consistent even when different types of neuromuscular blocking drugs have been tested. Notably, the hypercapnic ventilatory response is unaffected and thereby also the respiratory muscles. That means that this depression of HVR occurs at levels of neuromuscular

blockade that do not impair the mechanics of breathing or baseline ventilation, but rather through direct influence on control of breathing. In a series of animal studies, it has been demonstrated that this is primarily due to inhibition of carotid body oxygen signalling via nicotinic acetylcholine receptors at the cell-afferent nerve synapse [8, 9].

Obstructive sleep apnoea (OSA) has been identified as a common comorbidity in the peri-operative period and is also associated with an increased risk for peri-operative complications, including postoperative hypoxic events and re-intubation with unplanned admission to critical care [10–14]. Obstructive sleep apnoea is characterised by repetitive inspiratory upper airway collapse during sleep, causing partial or complete cessation of airflow. This leads to frequent periods of oxygen desaturation and arousal that ultimately lead to changes in regulation of breathing and surges of sympathetic activation [15]. As a consequence, HVR is increased in untreated OSA patients [16], and restored towards normal after 1 month of nightly home continuous positive airway pressure (CPAP) treatment [17]. Thus, OSA patients presenting for surgery may have different phenotypes with regard to regulation of hypoxic ventilation. It is plausible, therefore, that OSA patients respond differently to neuromuscular blockade with respect to respiratory control. If, for example, the augmented HVR in untreated OSA patients acts protectively in the face of residual neuromuscular blockade, we might need to be less concerned about respiratory depression. If, on the other hand, neuromuscular blockade acts synergistically with OSA to produce significant respiratory chemoreflex depression, then there is an increased cause for concern in the postoperative period. Therefore, the aims of this study – in a physiological setting – were to investigate the effect of a partial neuromuscular block on hypoxic and hypercapnic ventilatory responses in patients with untreated OSA.

Methods

The study conforms to the standard of the Declaration of Helsinki and was approved by the Regional Ethics Committee on Human Research at Karolinska Institute, Stockholm, Sweden and the Swedish Medical Products Agency, Uppsala, Sweden. Oral and written consent was obtained from all patients taking part in the study and the trial was conducted according to Good Clinical Practice.

This prospective, interventional trial was conducted at the Karolinska University Hospital, Stockholm, Sweden, between October 2012 and May 2014. Ventilatory tests were performed at the Karolinska University Hospital, and patients were recruited and diagnosed at an outpatient sleep clinic, (Aleris FysiologLab, Stockholm, Sweden) All eligible patients were consecutively asked to participate in the study.

Inclusion criteria were adult non-smoking males aged 18–70 years, body mass index (BMI) < 35 kg.m^{-2} and with newly diagnosed and untreated moderate to severe OSA scheduled for CPAP treatment. The only medical condition permitted, apart from OSA, was well-controlled hypertension with unchanged antihypertensive medication for the past 3 months. Exclusion criterion was allergy to rocuronium. In these patients, the diagnosis of OSA was made independently by sleep physicians in a separate medical team, using an in-home diagnostic sleep test (Embletta®, Embla, Broomfield, CO, USA) [18], providing continuous recording of thoracic and abdominal movements, nasal airflow through a nasal cannula connected to a pressure transducer, S_pO_2, pulse and body position through a built-in sensor. An obstructive apnoea was defined as a decrease in airflow by 90% of baseline for ≥ 10 s during continuous abdominal and thoracic movements according to the American Academy of Sleep Medicine [19]. A hypopnoea was defined as a decrease in airflow by 30% of baseline for a minimum of 10 s in combination with abdominal and thoracic movements and a decrease of oxygen saturation of at least 4%. The apnoea-hypopnoea index (AHI), i.e. mean number of events/h of sleep, was calculated from estimated total sleep time. The recordings were analysed manually by medical physicians specialised in sleep medicine. An AHI of 15–30 was considered as moderate OSA and AHI ≥ 30 as severe OSA.

Patients had no solid food intake for 6 h and no oral fluids for 2 h before the study. Alcohol and recreational drugs were not permitted for 24 h before the intervention. Antihypertensive medication was continued as normal. Patients were placed supine with a 30° head-up tilt. A facemask was tightly fitted with a dead space of 160–200 ml depending on the size of the face. A 20G intravenous (i.v.) catheter (Versatus-W, Terumo Europe NV, Leuven, Belgium) was inserted into a vein of the right arm for i.v. infusion of buffered crystalloid Glucos Braun (70 mM sodium, 45 mM chloride, 25 mM acetate, 2.5% glucose; B. Braun Melsungen AG, Melsungen, Germany) and later i.v. administration of rocuronium. Neuromuscular monitoring was performed on the contralateral arm. A schematic presentation of the test protocol is described in Fig. 1.

Isocapnic HVR and normoxic hypercapnic ventilatory response (HCVR) measurements were performed at four separate occasions separated by resting periods (Fig. 1). At the first occasion, HVR and HCVR were measured while breathing in an ordinary facemask without addition of CPAP at atmospheric pressure; the remaining three

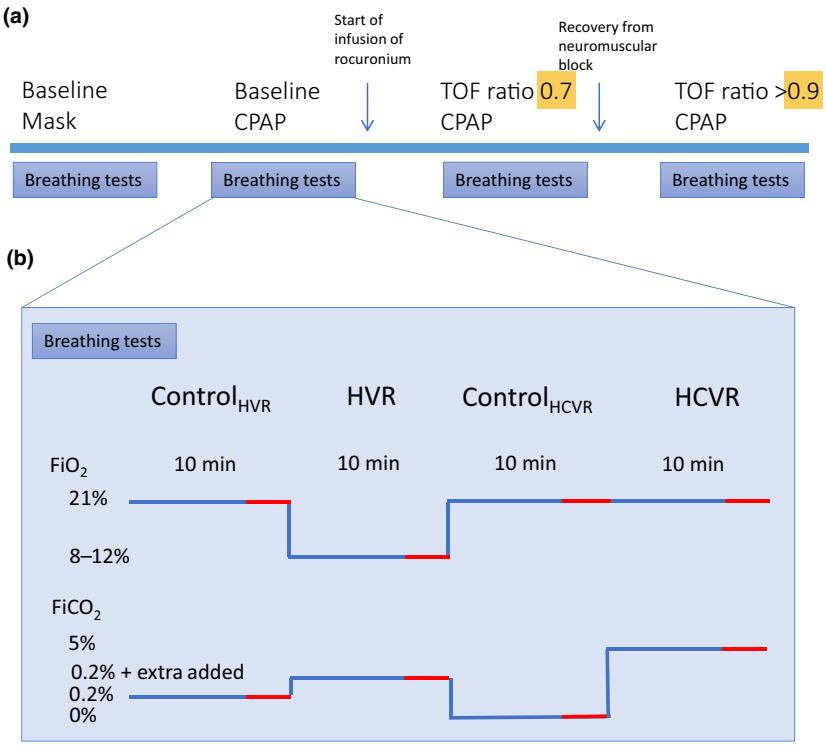

Figure 1 Schematic overview of the test protocol. The uppermost timeline shows the four study epochs (baseline mask breathing, followed by CPAP for the remainder of the study). Within each of these four epochs was a set of breathing tests, and the time courses of inspired gases is shown in outline in the inset panel. Each epoch consisted of 10 min of breathing air (with the addition of 0.2% CO_2 to the inspirate), followed by isocapnic hypoxia (isocapnia maintained by adding CO_2 to the inspirate); then 10 min air breathing; followed lastly by 10 min normoxic hypercapnia. Ventilatory measurements were made in the last 3 min of each of these 10 min period (shown in red). F_IO_2, inspired fraction of oxygen; F_ICO_2, inspired fraction of carbon dioxide; HVR, hypoxic ventilatory response; HCVR, hypercapnic ventilatory response; CPAP, continuous positive airway pressure.

occasions were performed during continuous CPAP breathing at 5 cmH_20 (Whisperflow® CPAP Valve, Respironics®, Wallingford, CT, USA) in order to ensure an open airway during partial neuromuscular block. The second occasion (with CPAP) served as a baseline recording before rocuronium administration, the third occasion was performed during rocuronium-induced partial neuromuscular block targeting an adductor pollicis TOF ratio of 0.7 and the fourth occasion was performed after recovery to a TOF ratio of > 0.9. The CPAP valve of 5 cmH_2O was removed for 2 min during the resting period before HCVR for the second, third and fourth test occasions to allow for observations of any upper airway obstructions. There were no signs of upper airway obstruction during the periods without CPAP and partial neuromuscular block.

After each period of ventilatory recordings (control, hypoxia, control and hypercapnia) the study subject was asked to report any perceived discomfort or sleepiness using a visual analogue scale ranging from 0 to 10. Zero represented no discomfort or fully awake while 10 represented maximum discomfort or asleep.

We planned to use the same protocol and set-up before and after 3 months of CPAP treatment, and data regarding the use of the home CPAP device (S9 AutoSet™, ResMed, Sydney, NSW, Australia) over 3 months was collected from the CPAP software (ResScan™, ResMed, Sydney, NSW, Australia).

All studies started at 08.00 am and took approximately 4 h to complete. Study subjects were not disturbed unless they were about to fall asleep and normal background noise was allowed under standard artificial room light.

Continuous standard peri-operative monitoring was used throughout the experimental period [20] and included ECG, peripheral oxygen saturation (S_pO_2), inspired fraction and end-tidal pressures of oxygen and carbon dioxide (F_IO_2, F_ICO_2, ETO_2 and ET_{CO_2}), respiratory rate and palmar temperature (Datex-Ohmeda AS/3™ and S/5™ Collect GE Medical systems Madison WI, USA). End-tidal partial pressure of CO_2, P_{ETCO_2}, airflow and inspired

and expired positive airway pressures were measured with a spirometer and tidal volumes were automatically calculated and recorded (D-lite™, Datex-Ohmeda AS/3™ and S/5™ Collect GE Medical systems Madison WI, USA). Non-invasive blood pressure was measured at least every 5 min (Datex-Ohmeda AS/3™ and S/5™ Collect GE Medical systems Madison WI, USA). Continuous thoracic and abdominal impedance was measured (Bio-Radio™, Great Lakes Neuro Technologies, Valley View, OH, USA).

An open breathing circuit (Engström 2024, Stockholm, Sweden) and two Optiflows™ (Fisher & Paykel Healthcare, Auckland, New Zealand) were combined and with a total flow of 155 $l.min^{-1}$. Calibration of the breathing system was done before each experiment with a 500 ml and 1000 ml calibration syringe. All volunteers started with a resting period breathing room air for a minimum of 15 min in order to adjust to the equipment. Consecutive resting periods were at least 6 min long. During resting ventilation preceding a hypoxic ventilatory test and at the end of the resting period, 0.2% carbon dioxide was added into the circuit.

During the isocapnic HVR test, there was a manually-controlled step introduction of nitrogen to achieve F_IO_2 of 0.1–0.12, targeting S_pO_2 of 80%. Carbon dioxide was added into the circuit with a micro-flow meter in order to maintain constant ET_{CO_2}. The hypoxic period was at least 10 min. The isocapnic HVR was measured for the last 3 min. The HVR was defined as:

$$\mathrm{HVR}(l \cdot \mathrm{min}^{-1} \cdot \%^{-1}) = \frac{\mathrm{VE\ hypoxia} - \mathrm{VE\ control}}{S_pO_2\ \mathrm{control} - S_pO_2\ \mathrm{hypoxia}} = \frac{\Delta \mathrm{VE}}{\Delta S_pO_2} \quad (1)$$

After the hypoxic test, the study subjects had at least 10 min of rest, breathing room air, before starting the hypercapnic test.

During normoxic HCVR, CO_2 was manually titrated into the circuit with a micro-flow meter to achieve F_ICO_2 of 5% in inspired air. The hypercapnic period was at least 10 min long. After reaching steady state measurements were made during the last 3 min. HCVR was calculated as:

$$\mathrm{HCVR}(l \cdot \mathrm{min}^{-1} \cdot \mathrm{kPa}^{-1}) = \frac{\mathrm{VE\ hypercapnia} - \mathrm{VE\ control}}{P_{ETCO_2}\ \mathrm{hypercapnia} - P_{ETCO_2}\ \mathrm{control}} = \frac{\Delta \mathrm{VE}}{\Delta P_{ETCO_2}} \quad (2)$$

Neuromuscular function was assessed by recording of the mechanical adductor pollicis muscle TOF response after supramaximal ulnar nerve stimulation using mechanomyography (Myograph 2000®, Biometer, Odense, Denmark) according to international consensus guidelines on good neuromuscular research practice [21] and as previously described [5–7, 22]. In short, surface electrodes were placed above the ulnar nerve and connected to a Myotest® nerve stimulator (Biometer, Odense, Denmark) and set at 1 Hz single-twitch stimulation. Supramaximal ulnar nerve stimulation was obtained by increasing the current 15–20% above the maximal response. The stimulation was then changed from single-twitch to TOF (0.3 ms impulses at 2 Hz for 2 s every 12 s) with a preload kept at 0.25–0.3 kg. After a warming-up period of approximately 20 min, calibration was performed and the TOF stimulus pattern was used throughout the experiment. A temperature probe was taped into the palm of the hand and used for monitoring (Datex-Ohmeda AS/3™ and S/5™ Collect GE Medical systems Madison WI, USA) to assure that the hand temperature was kept above 32.0 °C by means of an external heat pad.

Rocuronium (Esmeron® 10 $mg.ml^{-1}$, Merck Sharp & Dohme, Harleem, the Netherlands) was diluted with saline to a concentration of 0.5 $mg.ml^{-1}$ and given as a continuous i.v. infusion using a standard syringe pump (Perfusor® Space, B Braun Melsungen AG, Melsungen, Germany). The infusion of rocuronium was started at 0.3 $mg.kg^{-1}.h^{-1}$, a starting rate that previously has been shown to be adequate [22]. Thereafter the infusion rate was adjusted in accordance with response to achieve and maintain TOF ratio 0.7.

The study was powered to detect a difference in HVR of at least 0.15 units (see Eqn 1) between HVR control and HVR at TOF 0.7 (two-tailed α error of 0.05 and a β error of 0.2; power 80%). Assuming that the standard deviation of the difference in the HVR effect was 0.15 (effect size $d = 1$) a sample size of 10 was suggested.

Cardiorespiratory data from each experimental period were averaged over the last 3 min of recording, i.e. during steady-state ventilation (Fig. 1). For the primary and secondary aim, baseline HVR and HCVR with CPAP were compared with HVR and HCVR when TOF ratio was 0.7. Comparisons for HVR and HCVR were analysed using Wilcoxon signed rank test. Statistical analysis and graphs were made using Prism 6.0 (GraphPad, Software Inc., La Jolla, CA, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Ten male study subjects, mean (SD) age 52.2 (15.0) y, BMI 29.9 (2.8) kg.m^{-2}, AHI 30.0 (13.6) and oxygen desaturation index 25.5 (11.4) were included in the study. Nine of these untreated study subjects conducted the HVR tests, however in one patient the HVR test at TOF ratio 0.7 had to be excluded due to technical reasons and in another study subject the HVR at baseline with CPAP had to be replaced with the HVR at baseline with facemask (see also Supporting Information, Figure S1).

Only three study subjects were able to complete the entire protocol. One subject only participated in baseline experiments, one dropped out after measurement of HVR at TOF 0.7, and four dropped out after HVR measurement at TOF > 0.9, leaving eight subjects who completed most of the protocol. Patients found the hypercapnic ventilatory tests distressing (so only three subjects completed the last epoch of these), and they also perceived discomfort from the mechanomyograph. We noted that there were several episodes with irregular breathing during the experiment (see also Supporting Information, Figure S2) and so avoided measurements during these periods. Hence, although in our protocol (Fig. 1) intended timings were 10 min for each period during breathing tests, actual timings varied slightly.

The targeted TOF ratio was 0.7 and the maintained steady-state adductor pollicis TOF ratio was mean (SD) 0.74 (0.03) before and 0.75 (0.03) during measurement of HVR and 0.77 (0.05) before and 0.70 (0.07) during HCVR in untreated OSA patients. The subjects received mean (SD) 0.36 (0.08) mg.kg^{-1} of rocuronium and it took 32.3 (14.6) min to reach the predefined TOF ratio of 0.7. After discontinuation of rocuronium infusion, the subjects had at TOF ratio > 0.90 in mean (SD) 4.5 (1.8) min. A summary of neuromuscular block and administration of rocuronium in the OSA patients that participated in the study before and after CPAP treatment are shown in the Supporting Information (Table S1).

The results of ventilatory measurements for the hypoxic tests within each epoch (Fig. 2a) are shown in Table 1. The target S_pO_2 was attained during each breathing test, and isocapnia suitably maintained. HVR was clearly attenuated during the period of TOF ratio 0.7, with a reduction of the HVR by 32.2% (p = 0.016) compared with the HVR CPAP control epoch. The HVR after recovery of the TOF ratio to > 0.90 was no different from control CPAP epoch measurement (Fig. 2a).

The results of ventilatory measurements for the hypercapnic tests within each epoch (Fig. 2b) are shown in Table 2. A suitably equivalent hypercapnic stimulus was achieved within each of the four epochs. The HCVR was unchanged during TOF ratio 0.7 compared with the HCVR CPAP control epoch (p = 0.250), and was maintained after recovery to TOF ratio > 0.9 (Fig. 2b).

There was no difference in HVR and HCVR during baseline breathing tests without or with CPAP (see also Supporting Information, Figure S3a and b).

Incidental cardiovascular data are presented in online supplementary files. Heart rate appeared to increase with hypoxic and hypercapnic stimulus, as well as rise throughout the course of the experiment (see also Supporting Information, Figure S4a). Diastolic blood

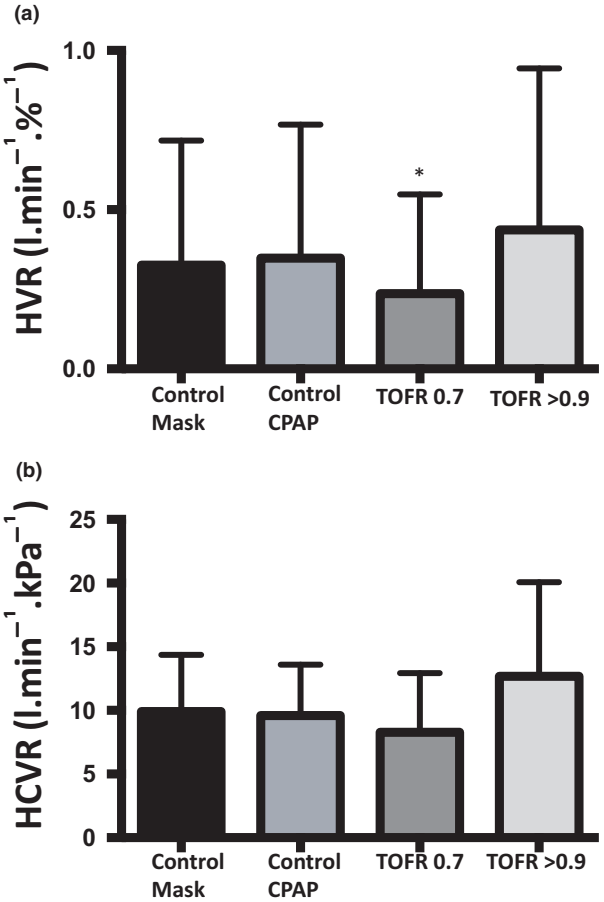

Figure 2 (a) HVR during each of the four epochs of Fig. 1. Data are mean (SD) with n = 8 for control and TOF ratio 0.75 and n = 6 for TOF ratio > 0.9; *p = 0.016 between control and TOF ratio 0.75. (b) HCVR during each of the four epochs of Figure 1. Data are mean (SD) with n = 8 for control and TOF ratio 0.70 and n = 3 for TOF ratio > 0.9. HVR, hypoxic ventilatory response; HCVR, hypercapnic ventilatory response; TOF, train-of-four.

Table 1 Summary of respiratory variables recorded during the last 3 min of each hypoxic exposure during each of the four epochs in Fig. 1 of the protocol. Values are mean (SD).

	Baseline mask n = 8	Baseline CPAP n = 8	TOF ratio 0.7 n = 8	TOF ratio > 0.9 n = 6
VE; l.min^{-1}				
Normoxia	10.3 (2.7)	8.8 (1.6)	9.3 (1.1)	9.7 (0.6)
Hypoxia	13.4 (3.1)	12.8 (3.2)	11.9 (2.7)	14.1 (3.7)
Δ VE	3.1 (3.1)	4.0 (4.0)	2.6 (3.1)	4.4 (4.3)
Respiratory rate; breaths.min^{-1}				
Normoxia	12.2 (3.4)	13.0 (3.0)	14.3 (4.2)	13.3 (3.8)
Hypoxia	12.9 (3.4)	13.4 (2.0)	13.9 (3.9)	12.5 (4.2)
Δ respiratory rate	0.6 (1.0)	0.4 (1.3)	−0.4 (2.0)	−0.8 (2.4)
Vt; ml				
Normoxia	919 (370)	728 (241)	693 (200)	774 (173)
Hypoxia	1122 (425)	998 (364)	934 (375)	1230 (656)
Δ Vt	203 (188)	276 (331)	232 (350)	461 (519)
P_{ETCO_2}; kPa				
Normoxia	4.5 (0.7)	4.2 (0.6)	4.3 (0.5)	4.1 (0.4)
Hypoxia	4.5 (0.6)	4.4 (0.5)	4.2 (0.4)	4.3 (0.4)
Δ P_{ETCO_2}	0.0 (0.2)	0.1 (0.1)	0.0 (0.2)	0.1 (0.2)
S_pO_2; %				
Normoxia	95.4 (1.9)	96.1 (0.8)	96.2 (0.5)	96.4 (0.9)
Hypoxia	81.6 (3.9)	81.5 (4.1)	82.6 (3.2)	82.9 (4.1)
Δ S_pO_2	13.88 (3.9)	14.6 (3.7)	13.6 (3.1)	13.5 (4.2)
EtO_2; %				
Normoxia	15.4 (1.4)	16.0 (1.0)	15.6 (0.8)	15.8 (0.6)
Hypoxia	7.4 (0.7)	7.4 (0.5)	7.3 (0.5)	7.2 (0.78)
Δ EtO_2	8.0 (1.4)	8.6 (0.8)	8.3 (0.9)	8.6 (0.9)
TOF ratio achieved	0.98 (0.05)	0.99 (0.03)	0.75 (0.03)	1.00 (0.02)

HVR, hypoxic ventilatory response; TOF, train-of-four; VE, minute ventilation; Vt, tidal volume; P_{ETCO_2}, partial pressure of end-tidal carbon dioxide; S_pO_2, peripheral oxygen saturation; EtO_2, End-tidal oxygen concentration; CPAP, continuous positive airway pressure.

pressure showed similar trend to the heart rate, with slightly higher values during hypoxia and hypercapnia whereas the systolic blood pressure increased slightly only during hypercapnia (see also Supporting Information, Figure S4b).

Patients reported most 'sleepiness' during the TOF ratio 0.7 epoch and felt breathing discomfort (dyspnoea) during all of the hypercapnic tests (see also Supporting Information, Figure S4c and d). All patients felt the cutaneous ulnar nerve TOF stimulation to be painful. Three patients reported double vision during partial neuromuscular block, that quickly disappeared when the rocuronium infusion was stopped. No other symptoms of the partial neuromuscular block, were reported.

Four OSA patients repeated the experiments after 3 months of CPAP treatment. The HVR results are shown in the Supporting Information (Figure S5a and Table S2). The HCVR results are shown in the Supporting Information (Figure S5b and Table S3).

Discussion

In context of our initial hypothesis, the main result of this study is that partial neuromuscular blockade in untreated patients with OSA reduced HVR to broadly the same extent as in healthy volunteers. Clinical studies show an increase in HVR in untreated OSA patients [16, 23, 24] and are supported by preclinical data showing hyperplasia and up-regulation of the hypoxic sensitivity of the carotid body [25, 26] in intermittent hypoxia. However, this is not protective against the effects of NMBD. Our experimental technique helped ensure that these results were not artefact due to worsening of airway obstruction by hypoxia, or by NMBD [27]. First, there was no difference in HVR between the mask breathing and CPAP epoch (the latter maintaining airway

Table 2 Summary of respiratory variables recorded during the last 3 min of each hypercapnic exposure during each of the four epochs in Fig. 1 of the protocol. Values are mean (SD).

	Baseline mask (n = 8)	Baseline CPAP (n = 8)	TOF ratio 0.7 (n = 8)	TOF ratio > 0.9 (n = 3)
VE; l.min^{-1}				
Normoxia	9.0 (2.0)	8.4 (1.3)	9.6 (2.2)	8.6 (1.8)
Hypoxia	23.5 (6.2)	25.1 (3.2)	24.9 (4.6)	26.2 (3.0)
Δ VE	14.4 (6.0)	16.5 (3.B)	15.3 (5.8)	17.6 (4.4)
Respiratory rate; breaths.min^{-1}				
Normoxia	11.2 (3.8)	11.1 (2.6)	14.3 (4.2)	10.8 (3.1)
Hypoxia	14.4 (2.9)	15.1 (3.5)	15.5 (4.2)	16.7 (4.6)
Δ respiratory rate	3.2 (2.4)	3.9 (2.6)	1.3 (3.0)	5.9 (6.9)
Vt; ml				
Normoxia	883 (285)	788 (179)	704 (201)	814 (124)
Hypoxia	1729 (440)	1753 (265)	1682 (485)	1617 (315)
Δ Vt	823 (405)	964 (294)	978 (517)	803 (420)
P_{ETCO_2}; kPa				
Normoxia	4.3 (0.8)	4.2 (0.6)	3.9 (0.6)	4.5 (0.6)
Hypoxia	6.1 (0.4)	6.1 (0.3)	5.9 (0.3)	6.1 (0.2)
Δ P_{ETCO_2}	1.8 (0.5)	1.8 (0.4)	2.1 (0.5)	1.6 (0.5)
S_pO_2; %				
Normoxia	95.1 (1.5)	96.2 (1.3)	96.3 (0.9)	95.6 (2.4)
Hypoxia	97.6 (0.8)	97.7 (0.7)	97.6 (0.7)	97.6 (0.5)
Δ S_pO_2	−2.5 (1.4)	−1.5 (1.3)	−1.4 (0.8)	−2.0 (1.9)
EtO_2; %				
Normoxia	15.7 (0.8)	15.9 (1.2)	16.5 (0.9)	15.9 (2.3)
Hypoxia	18.2 (1.2)	18.5 (1.3)	18.8 (1.6)	19.9 (2.2)
Δ EtO_2	−2.5 (1.0)	−2.6 (0.8)	−2.2 (1.7)	−4.0 (0.6)
TOF ratio achieved	0.99 (0.04)	0.99 (0.02)	0.70 (0.07)	1.00 (0.04)

HCVR, hypoxic ventilatory response; TOF, train-of-four; VE, minute ventilation; Vt, tidal volume; P_{ETCO_2}, partial pressure of end-tidal carbon dioxide; S_pO_2, peripheral oxygen saturation; EtO_2, end-tidal oxygen concentration; CPAP, continuous positive airway pressure.

patency). Second, partial paralysis did not impair HCVR (Table 2 and Fig. 2b) but only the HVR (Table 1 and Fig. 2a). Thus, patients were able to mount a robust ventilatory response to some chemostimulants, and the effect of NMBD was specific to the hypoxic chemoreflex.

Before we consider the implications of these results, we will address some limitations – and strengths – of our study. Although the hypoxic and hypercapnic stimuli we used were rapid, we did not employ a computer-controlled dynamic end-tidal forcing technique. Previous work has suggested that slow induction of hypoxia can result in different ventilatory responses as compared with more rapid step changes [28]. Similarly, it has been shown that the manner in which the hypercapnic stimulus is applied can influence ventilatory measurements [29] and that HCVR is influenced by how long the hypercapnic stimulus is sustained [30]. Nevertheless, end-tidal gas control was good and ventilatory responses reached steady states, so while this may affect any direct comparison of our results with other studies, it did not influence our measurements since the same stimulus types were employed across all experimental epochs (Fig. 1).

We noted that the level of discomfort from ulnar nerve stimulation was higher in this study compared with previous studies conducted by us [5–7, 22, 31, 32]. We acknowledge that pain can potentially influence measured ventilatory responses [33]. Furthermore, OSA patients showed an increased temporal stress during the conduction of the experiment, objectively displayed by an increased heart rate and blood pressure especially at the end of the experiment. Thus, only three patients completed the HCVR test at TOF > 0.9, so we cannot exclude that this stress response, perhaps compounded by repeated painful stimuli, may have been influential.

We targeted TOF ratio to 0.7 but this was not quite achieved. Moreover, there was a small difference in TOF ratios between the measure of HVR and HCVR (Tables 1 and 2) and this is because of the time differences at which these measurements were made (see Fig. 1). These differences were not of a magnitude that could explain the very different influence of neuromuscular blockade on HVR and HCVR but it may reflect the sensitivity of OSA patients' neuromuscular junction to these drugs. This might be a plausible explanation as previous work has suggested changes in neuromuscular function with OSA and CPAP [34].

We had intended also to assess the influence on these results of sustained treatment of OSA with CPAP, but recruited only a small number of patients to this part of the study. We present the results we obtained in online supplementary material (see also Supporting Information, Figure S3a and b and Tables S2 and S3). This preliminary data indicates that HVR during partial neuromuscular block is depressed to a similar magnitude after 3 months of CPAP treatment as before treatment. But this must be regarded as a provisional conclusion, which would be important to pursue in further investigation.

Our main result of a specific depression of HVR but not HCVR with partial neuromuscular blockade is consistent with previous work and as previously shown [8, 9, 35–38] is most likely due to an inhibition of synaptic acetylcholine-dependent nicotinic chemotransmission within the carotid body. This is interesting because such separation of effects on hypoxic and hypercapnic stimuli usually implies a drug effect directly on the carotid body type-1 glomus cell. However, at the synapse and pathway beyond in the afferent nerve, there is convergence of hypoxic and hypercapnic stimuli [39]. Yet, O'Donohoe et al. have recently demonstrated that neuromuscular blocking drugs applied directly to the isolated glomus cell does not inhibit its hypoxic response [14]. Therefore, we conclude that the maintenance of HCVR in our OSA subjects is likely due to persistent central chemoreceptor response.

Although ours is a physiological study, there are important clinical implications. The results underline the additional risk faced by OSA patients if there is residual neuromuscular blockade. Not only are they at risk from their underlying disease, but partial paralysis also inhibits their HVR. An encouraging finding was that the HVR was largely restored through recovery from paralysis (Table 1 and Fig. 2a). We used rocuronium and since there is the opportunity to reverse the effects of this rapidly and near-completely with sugammadex [40], this suggests reversal of neuromuscular blockade is also important for restoration of depressed chemoreflex function.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Individual contribution of each study subject before and after continuous positive airway pressure (CPAP) treatment.

Figure S2 Irregular breathing patterns in untreated obstructive sleep apnoea patients.

Figure S3 The hypoxic and hypercapnic ventilatory responses (HVR and HCVR) during ordinary facemask and continuous positive airway pressure (CPAP) of 5 cmH_20 in untreated obstructive sleep apnoea patients.

Figure S4 Cardiovascular data across the four experimental epochs (Figure 1).

Figure S5 (a) The hypoxic and hypercapnic ventilatory responses (HVR and HCVR) during partial neuromuscular block after three months of nightly continuous positive airway pressure treatment.

Table S1 Summary of neuromuscular block and administration of rocuronium in obstructive sleep apnoea patients before and after three months of home continuous positive airway pressure treatment.

Table S2 Summary of respiratory variables recorded during the last 3 min of each hypoxic exposure during each of the four epochs in Figure 1 of the protocol in the four obstructive sleep apnoea patients that participated after three months of nocturnal continuous positive airway pressure treatment.

Table S3 Summary of respiratory variables recorded during the last 3 min of each hypercapnic exposure during each of the four epochs in Figure 1 of the protocol in the three obstructive sleep apnoea patients that participated after three months of nocturnal continuous positive airway pressure treatment.