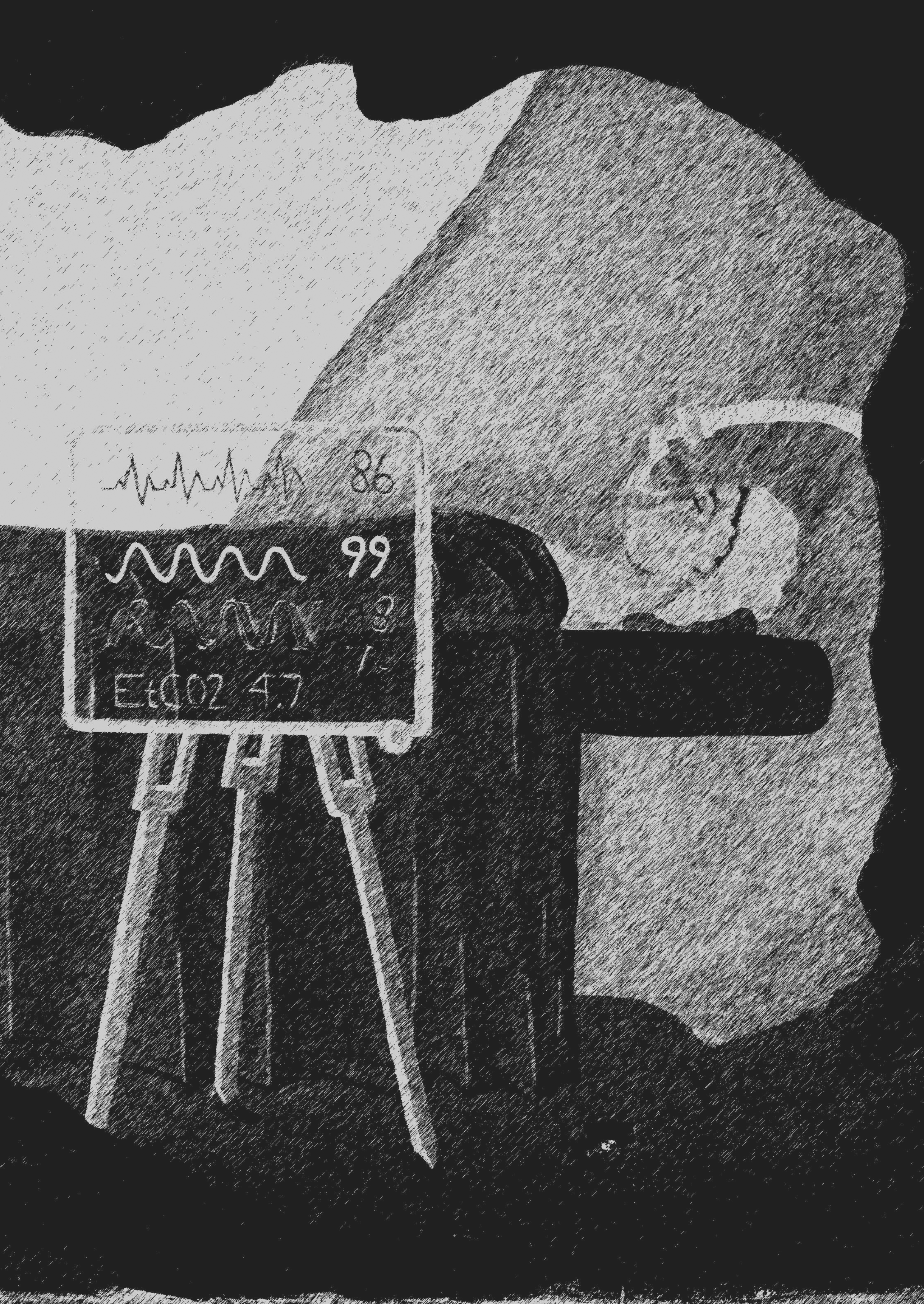
Monitoring anesthesia: Optimizing monitoring strategies to reduce adverse effects of anesthetic drugs on ventilation

Broens, S.J.L.

Author: Broens, S.J.L.
Title: Monitoring anesthesia: Optimizing monitoring strategies to reduce adverse effects of anesthetic drugs on ventilation
Issue Date: 2020-12-01


86
99
EtCO2 4.7

Section 2

Monitoring of Neuromuscular Block

CHAPTER 4

4

Influence of reversal of a partial neuromuscular block on the ventilatory response to hypoxia: a randomized controlled trial in healthy volunteers

Broens SJL, Boon M, Martini CH, Niesters M, van Velzen M, Aarts LPHJ, Dahan A.

Anesthesiology. 2019 Sep;131(3):467-476

Introduction

Residual neuromuscular blockade, defined by a train-of-four ratio less than 0.9, is associated with impaired function of respiratory and pharyngeal muscles with an increased risk of hypoventilation, hypoxia, upper-airway obstruction, and pulmonary aspiration(1–5). Importantly, Eikermann *et al.*(2) demonstrated that even when the train-of-four ratio fully recovered to unity, respiratory function tests (*e.g.*, forced vital capacity) may still be depressed in some patients. There is now robust evidence that nondepolarizing neuromuscular blocking agents additionally influence ventilatory control by acting within the peripheral chemoreflex loop at the carotid bodies(6–11). The carotid bodies are located at the bifurcation of the common carotid artery and are important sensors involved in maintaining respiratory homeostasis(12). For example, in case of hypoxia, carotid body activation results in hyperventilation aimed at increasing pulmonary oxygen uptake. The carotid body response to hypoxia (the acute hypoxic ventilatory response) is a life-saving reflex that is impaired by various drugs used in the perioperative phase, including opioids and anesthetics(13,14). In two pivotal studies in the early 1990s, Eriksson *et al.*(6,7) showed in humans that the nondepolarizing neuromuscular blocking agent vecuronium at a train-of-four ratio of 0.7 blunts the acute hypoxic ventilatory response by 15 to 60%. Because the acute hypoxic ventilatory response was significantly more depressed than the *hyperoxic* ventilatory response to carbon dioxide (which is a central chemoreflex response), an effect of vecuronium on the peripheral chemoreflex loop seems most likely. Animal studies give further proof for a selective effect of neuromuscular blocking agents at neuronal nicotinic acetylcholine receptors located postsynaptically on the afferent nerve that connects the oxygen sensing or glomus cells of the carotid bodies to the brainstem(9–11). Eriksson(8) showed later similar results for atracurium and pancuronium and additionally demonstrated that spontaneous return of the train-of-four ratio to values greater than 0.9 resulted in the recovery of acute hypoxic ventilatory response to values not different from control, although some subjects showed persistent depression of the hypoxic response. The picture that emerges from human and animal data from the Eriksson research group is that nondepolarizing neuromuscular blocking agents, irrespective of their chemical structure or receptor affinity, significantly impair the peripheral chemoreflex at the carotid bodies at train-of-four ratios less than 0.9. So far, no other laboratories replicated Eriksson's human studies. The aim of our study was twofold. We replicated the concept of the human studies originally performed by Eriksson *et al.*(6–8) with rocuronium to confirm their results for a nondepolarizing neuromuscular blocking agent not yet studied. Additionally, we measured the acute hypoxic ventilatory response, the hyperoxic hypercapnic ventilatory response after recovery of the partial neuromuscular block (as measured at the thumb), following reversal with neostigmine and sugammadex *versus* placebo. We hypothesized that the carotid body–mediated hypoxic ventilatory response is fully restored following the return to a train-of-four ratio of 1, irrespective of reversal strategy.

Materials and Methods

Ethics

This single-center, double-blind, parallel, randomized controlled trial was performed at the Anesthesia and Pain Research Unit of the Department of Anesthesiology at Leiden University Medical Center (Leiden, The Netherlands) from May 2017 to September 2018. The protocol was approved by the local institutional review board (Commissie Medische Ethiek, Leiden, The Netherlands) and the Central Committee on Research Involving Human Subjects in The Hague, The Netherlands. The study was registered at the trial register of the Dutch Cochrane Center (Amsterdam, The Netherlands) under identifier 6427 (May 4, 2017). Before enrollment and after being informed about the study, all participants gave written informed consent. All study procedures were conducted according to good clinical practice guidelines and adhered to the tenets of the Declaration of Helsinki.

Subjects

Healthy male volunteers aged 18 yr or older and a body mass index less than 30 kg/m2 were eligible to participate in the study. Exclusion criteria were (1) known or suspected neuromuscular disorders impairing neuromuscular function; (2) suspected allergies to muscle relaxants, anesthetics, or narcotics; (3) a history (self or family) of malignant hyperthermia or any other muscle disease; (4) any medical, neurologic, or psychiatric illness (including a history of anxiety); (5) inability to give informed consent; and (6) signs (or history) of a possible difficult intubation. Subjects were asked not to eat or drink for at least 8 h before dosing with rocuronium. The subjects were not allowed to participate more than once in the study. The subjects were recruited and enrolled in the study by the study team.

Study Design

This study had a randomized, double-blind, placebo-controlled parallel design. Upon arrival in the research unit, all subjects received an intravenous line for administration of study drugs. Participants were randomized to receive placebo (2 ml of normal saline), 1 mg of intravenous neostigmine (combined with 0.5 mg of atropine), or 2 mg/kg sugammadex, following a continuous rocuronium infusion for 90 to 120 min aimed at a train-of-four ratio of 0.7. Before and during the rocuronium infusion, acute hypoxic and hyperoxic hypercapnic tests were performed. After the rocuronium infusion was stopped and a reversal agent was administrated, another set of ventilatory tests was performed. Throughout the study the subjects were monitored by electrocardiogram and oxygen saturation *via* a finger probe.

Initially, we had set out to obtain respiratory responses at two levels of neuromuscular blockade with train-of-four ratios 0.6 and 0.8. Especially at the deeper level of relaxation, we observed frequent upper-airway obstructions that, although short-lived and not hazardous, interfered with the control of end-tidal carbon dioxide and oxygen concentrations. We therefore decided to change the protocol to studying just one level of blockade in between the original targets. Additionally, we originally intended to study repetitive hypoxic exposures following reversal to measure the acute hypoxic ventilatory response at increasing train-of-four ratio values (from 0.7 to 1). However, we observed that the train-of-four ratio returned rather rapidly toward 1 and therefore decided to obtain one measurement of acute hypoxic ventilatory response at the time point at which the train-of-four ratio was first equal to 1. All changes were made after consultation with the data safety committee, were approved by the institutional review board, and documented in the trial registry.

Ventilatory Measurements

During ventilatory measurements, subjects were in the semirecumbent position. The ventilatory responses to hypoxia and hypercapnia were obtained using the dynamic end-tidal forcing technique. The technique is described in detail elsewhere.15,16 In brief, subjects breathed through a facemask that was attached to a pneumotachograph and pressure transducer system (catalog no. 4813; Hans Rudolph Inc., USA) and to a set of mass flow controllers (Bronkhorst High Tech, The Netherlands) for the delivery of oxygen, carbon dioxide, and nitrogen. The mass flow controllers were controlled by a computer running software (RESREG/ACQ, Leiden University Medical Center, The Netherlands) that steers end-tidal gas concentrations (by varying the inspired concentration) and collects respiratory variables. The inspired and expired oxygen and carbon dioxide partial pressures were measured at the mouth using a capnography (Datex Capnomac, Finland); heart rate and arterial oxygen saturation were measured by pulse oximetry (Masimo Corporation, USA). The following variables were collected on a breath-to-breath basis and averaged over 1 min for further analysis: minute ventilation (V_E), end-tidal carbon dioxide concentration (ET_{CO_2}), end-tidal oxygen concentration (ET_{O_2}), and arterial oxygen saturation (SpO_2). To obtain the isocapnic hypoxic ventilatory response, we performed steps into hypoxia by lowering the ET_{O_2} to 52 mmHg such that the SpO_2 dropped to 80± 2%. The hypoxic test took about 7 to 9 min, *i.e.*, 2 to 4 min of normoxia (ET_{O_2} 100 mmHg) followed by 5 min of hypoxia. Throughout the experiment the ET_{CO_2} was kept constant at 1 to 2 mmHg above resting values. To obtain the hyperoxic hypercapnic ventilatory response, we applied three 5- to 7-min steps in ET_{CO_2} with step sizes of 7.5, 10, and 15 mmHg. To suppress the contribution of the carotid bodies to the hypercapnic response, all hypercapnic tests were performed in hyperoxia (inspired oxygen fraction, 0.5).15 The hypoxic ventilatory sensitivity was calculated by linear regression of the SpO_2–V_E data using the last 2 min of normoxia and hypoxia. The hypercapnic ventilatory sensitivity was calculated by linear regression of the ET_{CO_2}–V_E data using the last 2 min of each hypercapnic step. This analysis provided the slope of the hypercapnic ventilatory response (S) and the extrapolated *x*-axis intercept (apneic threshold, B). Next, we calculated ventilation at an extrapolated ET_{CO_2} of 55 mmHg (V_E55) using the following equation $V_E55 = S \times (55 - B)$. Ventilation at an extrapolated ET_{CO_2} of 55 mmHg takes the slope and the position of the hypercapnic ventilatory response into account and hence gives a reliable reflection of the effect of the intervention on hypercapnic ventilatory control (see fig. 2 of van der Scrier *et al.*).17 To ensure that responses were unaffected by previous responses, we allowed rest periods between measurements. Additionally, minute ventilation was assessed in real time on-screen, and only when ventilation had returned to baseline levels was the next ventilatory measurement initiated.

Experiments were performed at baseline (before any drug administration), at a stable neuromuscular block with train-of-four ratio equals 0.7, and after recovery to a train-of-four ratio equals 1. The sequence of hypoxic and hypercapnic tests was randomized at baseline and during rocuronium infusion; however, following return of the train-of-four ratio to 1 after reversal, first one hypoxic test was obtained followed by the hypercapnic test.

Drug Administration

All drugs were given intravenously. For rocuronium (Esmeron, MSD BV, The Netherlands), the dosing was dependent on the measured train-of-four ratio. In all subjects the initial bolus dose was 5 mg, after which a rocuronium continuous infusion was started at 0.42 mg/min. Additional bolus doses of 1 to 5 mg were given, and/or the infusion rate was modified when the train-of-four ratio remained above the target. In case of an overshoot with train-of-four ratios of less than 0.7, the infusion was lowered until the target was reached. At the desired train-of-four ratio target, we waited 10 to 15 min, and when the train-of-four ratio remained stable, the first respiratory test was performed. Otherwise the infusion rate was further adapted until the target was reached. On average, 55± 15 mg rocuronium was given throughout the experiment. The rocuronium dosing was based on simulations using the pharmacokinetic data set of Kleijn *et al*.(18) The reversal agents 1 mg of neostigmine Hameln Pharmaceuticals Ltd., United Kingdom), 2 mg/kg sugammadex (Bridion, MSD BV), and 2 ml of placebo (NaCl 0.9%) were given as a bolus infusion when the rocuronium infusion was stopped. In case of an upper-airway obstruction or severe respiratory depression (with SpO_2 less than 70%) due to a more intense neuromuscular block than intended, the subject received 2 mg/kg sugammadex, and the experiment was ended.

Measurement of the Neuromuscular Block

Neuromuscular block was measured by electromyography at the adductor pollicis muscle, using the CARESCAPE B450 monitor combined with the electromyography–neuromuscular transmission module (both General Electric, Finland). After degreasing the skin with alcohol, the electrodes were placed at the wrist according to the guidelines of the manufacturer (in the arm opposite to the arm with the intravenous line). Before administration of rocuronium, a series of measurements were obtained at a stimulus strength of 30 mA. Stable recordings were verified and defined as a difference in train-of-four ratio of less than 5% in three consecutive measurements. All subsequent measurements were obtained at 1-min intervals. Skin temperature was maintained throughout the study by keeping a constant room temperature.

Randomization and Allocation

Randomization (placebo:neostigmine:sugammadex equals 1:1:1) was performed by an independent third party (research nurse not involved in the study) using a computer-generated randomization list. On the day of the experiment, each subject was allocated to treatment, and all study medication was delivered to the laboratory by the same nurse in unmarked sequentially numbered syringes of equal size and volume. In case of neostigmine administration, the syringe additionally contained 0.5 mg of atropine. The study was independently monitored, ensuring all Good Clinical Practices requirements were met.

Statistical Analysis

No formal sample size analysis was performed because we based our samples size on the previous studies of Eriksson *et al.*6–8 We defined the acute hypoxic response (AHR), slope of the hypercapnic ventilatory response (HCVR), and ventilation at an extrapolated ET_{CO_2} of 55 mmHg (V_E55) obtained at baseline as AHR_1, $HCVR_1$, and V_E55_1, respectively. Similarly, at a train-of-four ratio of 0.7, the responses are denoted AHR_2, $HCVR_2$, and V_E55_2, and after recovery of the neuromuscular block, the responses are denoted AHR_3, $HCVR_3$, and V_E55_3.

We calculated the ratio of acute hypoxic response and ventilation at an extrapolated ET_{CO_2} of 55 mmHg relative to their baseline values, with $AHR_{2R} = AHR_2/AHR_1$, $AHR_{3R} = AHR_3/AHR_1$, $V_E55_{2R} = V_E55_2/V_E55_1$, and $V_E55_{3R} = V_E55_3/V_E55_1$. Next, we calculated the ratios $F2 = AHR_{2R}/V_E55_{2R}$ and $F3 = AHR_{3R}/V_E55_{3R}$ as carotid body index (*i.e.*, markers of hypoxic chemosensitivity).(7) In some participants V_E55_3 values exceeded baseline ventilation at an extrapolated ET_{CO_2} of 55 mmHg values with consequently V_E55_{3R} values of more than 100%. We consider this excitatory phenomenon a procedural effect because the hypercapnic test after reversal was always performed after the hypoxic test. Because this may have underestimated the effect of reversal treatment on F3, we calculated correctedV_E55_{3R} and F3 values by constraining V_E55_{3R} to 100% in case values of more than 100% were observed.

All data were checked for normality by evaluation of their empirical distribution (*i.e.*, by histogram). The effects of treatment on acute hypoxic response, slope of the hypercapnic ventilatory response, and ventilation at an extrapolated ET_{CO_2} of 55 mmHg were analyzed by one-way ANOVA (factor: treatment) with *post hoc* Holm–Sidak multiple comparison test to compare treatment effect (either measurement 2 or 3) to control (baseline) data. The hypoxic and hypercapnic ratios F were compared to 1 using two-tailed paired *t* tests. These analyses were performed (using a two-tailed approach) on the complete data set and on the three distinct reversal treatments, allowing within-group comparison. To test our hypothesis that at a train-of-four ratio of 1 the responses fully returned to baseline levels irrespective of the reversal strategy, an analysis of covariance was performed on AHR3, HCVR3, VE553, with the baseline as covariate and treatment (placebo, neostigmine, sugammadex) as fixed effect. To compare the F3 ratios among treatments, a one-way ANOVA was performed. If a significant main effect was observed, protected *post hoc* tests were performed. The statistical analysis was performed using GraphPad Prism version 7 for Mac OS X (GraphPad Software, USA) and SPSS Statistics for Windows (IBM, USA), version 23.0. *P* values of less than 0.05 were considered significant. All values are means } SD unless otherwise stated; the data in the figures are means ± 95% CI.

Results

In the amended protocol 40 subjects were randomized (fig. 1). All subjects completed the protocol without serious adverse events. Four subjects developed upper-airway obstruction: three during the administration of rocuronium and one during recovery following placebo reversal. All four were treated with sugammadex, after which they fully recovered; they were taken out of the study, and each was replaced by another subject. The characteristics of the 36 subjects that completed the study are given in table 1.

Data from two subjects (one in the neostigmine group and the other in the sugammadex group) were unreliable due to lack of calibration. Consequently, the data from 34 subjects were analyzed. Apart from upper-airway obstruction, adverse effects included diplopia (80%), difficulty swallowing (40%), and ptosis (10%). After reversal, all subjects recovered fully. None of the subjects reported the occurrence of distress or anxiety during relaxation.

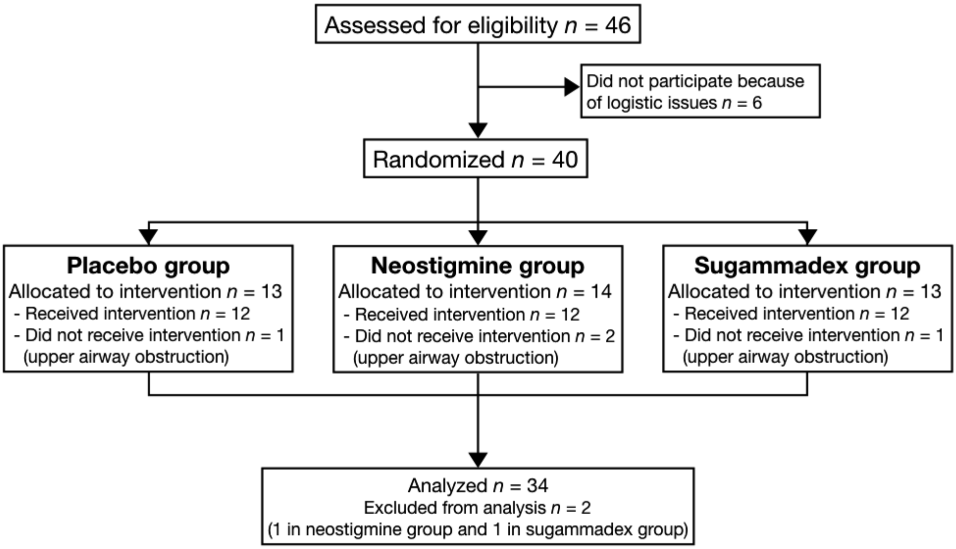


Figure 1. Consort flow diagram.

4

Table 1. Subject characteristics

		Reversal group		
	All subjects	**Placebo**	**Neostigmine**	**Sugammadex**
Age (median) [range]	22 [18-29] year	20 [19-29] year	23 [18-29] year	23 [19-25] year
Weight (mean ± SD)	80 ± 10 kg	79 ± 9 kg	77 ± 10 kg	86 ± 10 kg
Height (mean ± SD)	185 ± 8 cm	186 ± 10 cm	187 ± 9 cm	187 ± 8 cm
BMI (mean ± SD)	23.1 ± 2.1 kg·m^{-2}	22.6 ± 1.5 kg·m^{-2}	22.2 ± 2.1 kg·m^{-2}	24.7 ± 2.2 kg·m^{-2}

Influence of Low-dose Rocuronium and Reversal on Ventilatory Control

Upon the administration of rocuronium, the train-of-four ratio decreased slowly and reached a steady state of 0.68 ± 0.01 (fig. 2) within 45 min, after which the respiratory tests were performed. During the hypoxic tests, the ET_{CO_2} averaged to 41.1 ± 2.5 mmHg in control studies, 41.5 ± 2.6 mmHg in rocuronium studies, and 41.4 ± 2.6 mmHg in reversal studies. Hypoxic ventilator sensitivities during control, during relaxation, and after reversal were 0.55 ± 0.22 (AHR_1), 0.31 ± 0.20 (AHR_2), and 0.45 ± 0.16 (AHR_3) l · min–1 · %–1 (AHR_2 *vs.* AHR_1, $P < 0.001$; AHR_3 *vs.* AHR1, $P < 0.001$; table 2), respectively. AHR_2 was depressed by 42%, and V_E55_2 was depressed by 11% (tables 2 and 3).

Table 2. Acute Hypoxic Response and Slope of the Hypercapnic Ventilatory Responses Obtained at Baseline, during Infusion of Low-dose Rocuronium and after Reversal with Placebo, Neostigmine, or Sugammadex.

Treatment	**AHR_1** ($L.min^{-1}.\%^{-1}$)	**AHR_2** ($L.min^{-1}.\%^{-1}$)	**AHR_3** ($L.min^{-1}.\%^{-1}$)	**$HCVR_1$** ($L.min^{-1}.mmHg^{-1}$)	**$HCVR_2$** ($L.min^{-1}.mmHg^{-1}$)	**$HCVR_3$** ($L.min^{-1}.\%^{-1}$)
All treatments	0.55 ± 0.22	0.31 ± 0.20	0.45 ± 0.16	2.02 ± 0.66	1.51 ± 0.48	2.20 ± 0.96
Mean difference (95% CI)		-0.23 (-0.30 to -0.16) $p < 0.001$ vs. AHR_1	-0.10 (-0.15 to -0.05) $p < 0.001$ vs. AHR_1		-0.50 (-0.63 to -0.37) $p < 0.001$ vs. $HCVR_1$	0.19 (-0.02 to 0.40) $p = 0.094$ vs. $HCVR_1$
Placebo	0.66 ± 0.21	0.34 ± 0.22	0.49 ± 0.13	2.34 ± 0.69	1.68 ± 0.44	2.33 ± 0.88
Mean difference (95% CI)		-0.33 (-0.49 to -0.17) $p = 0.003$ vs. AHR_1	-0.18 (-0.26 to -0.10) $p = 0.002$ vs. AHR_1		-0.66 (-0.94 to -0.38) $p = 0.001$ vs. $HCVR_1$	-0.01 (-0.38 to 0.36) $p = 0.965$ vs. $HCVR_1$
Neostigmine	0.43 ± 0.23	0.29 ± 0.20	0.36 ± 0.20	1.81 ± 0.71	1.24 ± 0.47	2.05 ± 1.11
Mean difference (95% CI)		-0.15 (-0.20 to -0.10) $p < 0.001$ vs. AHR_1	-0.07 (-0.18 to 0.02) $p = 0.074$ vs. AHR_1		-0.56 (-0.77 to -0.35) $p = 0.001$ vs. $HCVR_1$	0.24 (-0.03 to 0.51) $p = 0.106$ vs. $HCVR_1$
Sugammadex	0.54 ± 0.16	0.31 ± 0.17	0.48 ± 0.13	1.88 ± 0.48	1.60 ± 0.47	2.24 ± 0.95
Mean difference (95% CI)		-0.23 (-0.29 to -0.17) $p < 0.001$ vs. AHR_1	-0.05 (-0.13 to 0.03) $p = 0.241$ vs. AHR_1		-0.27 (-0.47 to -0.07) $p = 0.004$ vs. $HCVR_1$	0.37 (-0.10 to 0.84) $p = 0.149$ vs. $HCVR_1$
Main treatment effect (ANCOVA)			$p = 0.299$			$p = 0.938$

The values are mean ± SD. AHR is the acute isocapnic hypoxic response; HCVR is the hyperoxic hypercapnic ventilatory response slope. AHR1 and HCVR1 are response obtained at baseline, AHR2 and HCVR2 are response obtained during infusion of rocuronium, AHR3 and HCVR3 are responses obtained following reversal. All within group statistical comparisons are relative to the baseline value (AHR1 or HCVR1). To assess the treatment effect on AHR3 and HCVR3, an analysis of covariance (ANCOVA) with baseline values (AHR1, HCVR1) as covariate and treatment as fixed effect was performed.

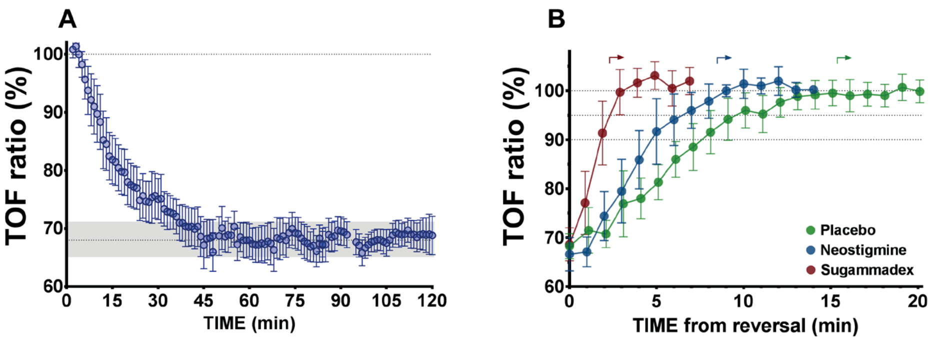


Figure 2. (**A**) Average train-of-four (TOF) ratio values during infusion of low-dose rocuronium (n = 36). Hypoxic and hypercapnic experiments were performed from t equals 45 to t equals 120 min (average TOF ratio, 68 ± 1%), after which reversal agents were given. (**B**) Effect of reversal with placebo (*green symbols*), neostigmine (*blue symbols*), and sugammadex (*red symbols*) on TOF ratios. The *arrows* indicate the mean times at which data collection of the hypoxic ventilatory response was started (hypoxic responses took 7 to 9 min). The values are means ± 95% CI.

Table 3. Ventilation at Extrapolated End-tidal Carbon Dioxide Concentration of 55 mmHg Measured at Baseline, during Low-dose Rocuronium Infusion and after Reversal with Placebo, Neostigmine, or Sugammadex.

Treatment	$VE55_1$	$VE55_2$	$VE55_3$
All treatments (L/min)	32 ± 9	28 ± 7	37 ± 11
Mean difference (95% CI)		-3.9 (-5.5 to -2.3) $p < 0.001$ vs. $VE55_1$	5.0 (3.5 to 6.5) $p < 0.01$ vs. $VE55_1$
Placebo (L/min)	35 ± 11	30 ± 8	39 ± 11
Mean difference (95% CI)		-5.5 (-9.1 to -1.9) $p = 0.020$ vs. $VE55_1$	3.7 (-1.0 to 8.4) $p = 0.159$ vs. $VE55_1$
Neostigmine (L/min)	29 ± 7	26 ± 7	34 ± 9
Mean difference (95% CI)		-3.0 (-5.0 to -1.0) $p = 0.026$ vs. $VE55_1$	4.9 (2.6 to 7.2) $p < 0.01$ vs. $VE55_1$
Sugammadex (L/min)	31 ± 8	27 ± 7	37 ± 14
Mean difference (95% CI)		-3.2 (-5.2 to -1.2) $p = 0.038$ vs. $VE55_1$	6.6 (-0.2 to 13.4) $p = 0.092$ vs. $VE55_1$
Main treatment effect (ANCOVA)			**p = 0.679**

The values are mean ± SD. VE55 is ventilation at an extrapolated extrapolated end-tidal carbon dioxide concentration of 55 mmHg. VE551 is hypercapnia ventilation measured at baseline, VE552 is hypercapnia ventilation measured during infusion of rocuronium, VE553 is hypercapnic ventilation measured following reversal. To assess treatment effect on VE553, an analysis of covariance (ANCOVA) with the baseline value as covariate (VE551) and treatment as fixed effect was performed.

Consequently, the effect of low-dose rocuronium on AHR_2 may be attributed largely to carotid body impairment with carotid body index F2 = 0.67 ± 0.32 (*P* < 0.001; table 4). Following reversal, AHR_3 was still depressed by 18%, but V_E55_3 was on average 15% greater than the value measured at baseline; V_E55_3 exceeded baseline values in 29 subjects. After adjustment for this excitatory effect, the corrected carotid body index F3 value averaged 0.89 ± 0.34 (*P* = 0.076).

Neostigmine, Sugammadex, and Placebo Reversal

Subject characteristics were similar among the three treatment groups (table 1). The train-of-four ratio recovery profiles for the three treatments are given in figure 2B. The times from reversal until the start of the hypoxic studies were 2.5 ± 0.7 (range 1 to 3) min, 8.2 ± 3.2 (4 to 16) min, and 15.1 ± 4.6 (12 to 25) min, for reversal with sugammadex, neostigmine, and placebo, respectively (*arrows* in fig. 3B). Although the magnitude of the control hypoxic responses among the three treatment groups varied (AHR_1 in table 2), these variations were not significantly different (*P* = 0.175), and partial relaxation by low-dose rocuronium resulted in a similar reduction by 38 to 46% (AHR_2) in the three treatment groups.

When considering the complete study population, reversal to a train-of-four ratio equals 1 led to an AHR3 of 0.45± 0.16 l · min−1 · %−1 (*P* < 0.001 *vs.* AHR_1) or a residual 18% depression compared to baseline. Within-group comparisons are given in table 2. The treatment effect (between-group comparison) did not reach the level of significance (analysis of covariance main effect *P* = 0.299), indicating that the three reversal strategies had a similar effect on the acute hypoxic ventilatory response. Relative to control levels, all treatments produced a similar increase in the hypercapnic ventilatory response (analysis of covariance main effect *P* = 0.938) and ventilation at an extrapolated ET_{CO_2} of 55 mmHg (analysis of covariance main effect *P* = 0.679; tables 2 and 3). Both uncorrected and corrected AHR3R values and corresponding F3 values are given in table 4 and figure 3. The uncorrected and corrected F3 values in the complete population were 0.78 ± 0.35 (*P* = 0.001) and 0.89 ± 0.34 (*P* = 0.076), respectively. Neither for the corrected nor for the uncorrected F ratios a significant treatment effect was observed (F3 uncorrected, ANOVA main effect *P* = 0.231; F3 uncorrected ANOVA main effect *P* = 0.232). Corrected F3 values less than 0.95 were observed in 10 subjects in the placebo group, 7 in the neostigmine group, and 5 in the sugammadex group.

Table 4. Ratios of the Acute Hypoxic Response (AHR) and Ventilation at an Extrapolated End-tidal Carbon Dioxide Concentration of 55 mmHg (VE55) Relative to Baseline (%) Obtained during Infusion of Low-dose Rocuronium (AHR2R and VE552R) and after Reversal with Placebo, Neostigmine, or Sugammadex (AHR3R and VE553R) and Carotid Body Indices Obtained during Low-dose Rocuronium Infusion (F2) and after Reversal (F3).

	Low-dose rocuronium (TOF-ratio = 0.7)			Reversal (TOF-ratio = 1)				
Treatment	**AHR_{2R}**	**$VE55_{2R}$**	**F_2**	**AHR_{3R}**	**$VE55_{3R}$** (uncorrected)	**$VE55_{3R}$** (corrected)	**F_3** (uncorrected)	**F_3** (corrected)
All treatments	57 ± 26	89 ± 11	0.66 ± 0.32	86 ± 25	115 ± 23	98 ± 7	0.78 ± 0.35	0.89 ± 0.34
Discrepancy (vs. 1) (95% CI)			-0.34 (-0.45 to -0.23) p < 0.001				-0.22 (-0.34 to -0.10) p = 0.001	-0.11 (-0.23 to 0.01) p = 0.076
Placebo	54 ± 33	85 ± 11	0.65 ± 0.43	76 ± 16	115 ± 24	97 ± 8	0.68 ± 0.16	0.79 ± 0.25
Discrepancy (vs. 1) (95% CI)			-0.34 (-0.62 to -0.06) p = 0.019				-0.32 (-0.42 to -0.22) p < 0.001	-0.21 (-0.31 to 0.11) P < 0.001
Neostigmine	62 ± 25	90 ± 12	0.69 ± 0.28	87 ± 27	117 ± 14	100 ± 0	0.75 ± 0.25	0.87 ± 0.27
Discrepancy (vs. 1) (95% CI)			-0.31 (-0.50 to -0.12) p = 0.004				-0.25 ± 0.16 p = 0.008	-0.13 (-0.30 to 0.04) p = 0.128
Sugammadex	56 ± 16	90 ± 12	0.64 ± 0.23	95 ± 29	112 ± 30	96 ± 8	0.93 ± 0.53	1.03 ± 0.5
Discrepancy (vs. 1) (95% CI)			-0.36 (-0.52 to -0.20) p < 0.001				-0.07 ± 0.35 p = 0.661	0.03 (-0.30 to 0.36) p = 0.842
Main treatment effect (anova)							**p = 0.231**	**p = 0.232**

The values are means ± SD. To assess treatment effect on the F ratios, a one-way ANOVA was performed. TOF, train of four.

4

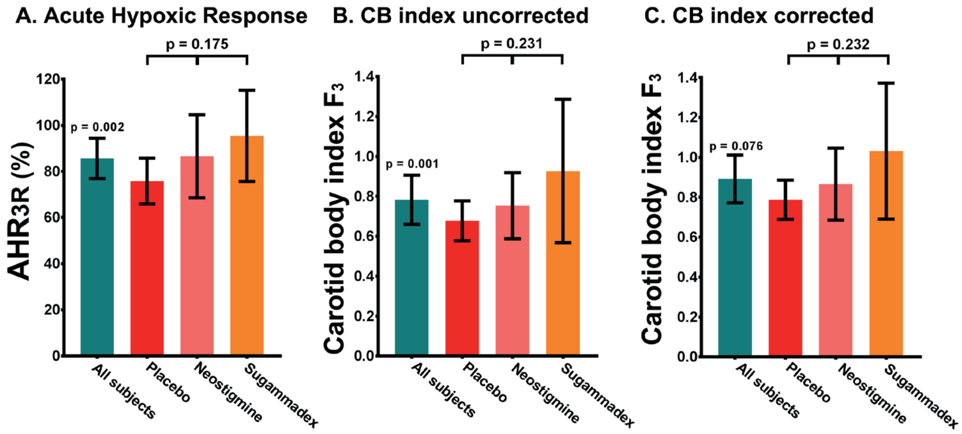


Figure 3. Influence of three reversal strategies on the acute hypoxic response and carotid body index F3. (**A**) AHR3R indicates the acute hypoxic ventilatory response after reversal as a percentage of the baseline response. (**B**) Uncorrected carotid body index F3, which is the ratios AHR3R/VE553R or the ratio of the changes in acute hypoxic response and ventilation at an extrapolated end-tidal carbon dioxide concentration of 55 mmHg (VE55), relative to baseline, after reversal and full recovery of neuromuscular function at the thumb. (**C**) Corrected carotid body index F3, with VE553R constrained to 100% in case of values exceeding 100%. The values are the means ± 95% CI. Between-treatment comparisons were done by analysis of covariance with baseline value as covariate. CB, carotid body.

Discussion

The main outcomes of our experimental study are summarized as follows:

1) Rocuronium-induced partial neuromuscular blockade (train-of-four ratio ≈ 0.7) blunts the isocapnic hypoxic response by 42%, whereas hypercapnic ventilation was reduced by just 11% (table 3). As estimated from the carotid body index (F2 = 0.67), the depression of the hypoxic response is primarily due to an effect on the peripheral chemoreflex loop. The additional depression of the hypoxic response is most probably related to mild respiratory muscle weakness.
2) Reversal of the neuromuscular block to a train-of-four ratio of 1 did not result in a complete return of the acute hypoxic response to baseline values (AHR_3 = 86 ± 25% of baseline, P = 0.002). We therefore reject the null hypothesis that full reversal of the neuromuscular block at the thumb results in a complete return of the acute hypoxic ventilatory response. Interestingly, this residual effect was independent of reversal strategy with 62% of subjects that still had AHR_3 values less than 95% of baseline (placebo n= 10, neostigmine n = 7, and sugammadex n = 5).

Partial Neuromuscular Blockade

In the first part of our study, we convincingly replicate the findings of Eriksson *et al.(*6–8) and show that partial neuromuscular block reduces the acute hypoxic response primarily *via* an effect at the carotid bodies. As proposed by Eriksson *et al.*(7), the separation between carotid body and muscle effects on ventilatory control was performed by calculating the carotid body index F2, which is the ratio AHR_{2R}/V_E55_{2R} or the ratio of relative changes in hypoxic response and ventilation at an extrapolated ET_{CO_2} of 55 mmHg. Because ventilation at an extrapolated ET_{CO_2} of 55 mmHg was obtained at hyperoxia, ventilation at an extrapolated ET_{CO_2} of 55 mmHg is minimally influenced by carotid body activity. Hyperoxia silences the contribution of the carotid bodies to hypercapnic ventilation, which is 10 to 20% under normoxic conditions *versus* 0 to 5% under hyperoxic conditions(15). Impairment of the hypoxic response *via* the respiratory muscles rather than carotid bodies would have resulted in carotid body index values not different from 1.7 The rocuronium F2 value of 0.66 (95% CI, 0.55 to 0.77) is smaller than the F value earlier observed for vecuronium (F = 0.84).7,8 This suggests a greater potency of rocuronium at impairing the carotid bodies compared with vecuronium.

4

Our data indicate a small non–carotid body–related effect on the hypoxic response during low-dose rocuronium infusion. Although this is most probably due to respiratory muscle weakness (illustrated by the decrease in V_E55_2), we cannot exclude other causes. Theoretically, one such cause could be a decrease in arousal level from deafferentation related to reduced muscle spindle input to central sites. There is ample evidence that peripheral deafferentation from spinal or epidural anesthesia changes the brain sensory and arousal states(19,20). A reduction in arousal level will decrease hypoxic response(14). Because we did not detect any obvious changes in arousal during partial muscle relaxation, we conclude that the non–carotid body–related effect of low-dose rocuronium on hypoxic response was related to mild respiratory muscle weakness. Although in line with the findings of Eikermann *et al.*(2) on rocuronium, our data differ from those of Eriksson *et al.*(6–8), who observed no effect of partial neuromuscular block on the slope of the hypercapnic ventilatory response. These differences may be attributed to differences in protocol or differences in drug sensitivity with greater rocuronium sensitivity in relaxation of respiratory muscles compared with other relaxants.

Reversal of the Partial Neuromuscular Blockade

The between-group comparison indicated that despite full reversal of partial neuromuscular block as evidenced by the measurement of the train-of-four ratio of 1 and a fully restored ventilation at an extrapolated ET_{CO_2} of 55 mmHg, impairment of the peripheral chemoreflex persisted in the majority of subjects, irrespective of the reversal strategy. It is further of interest to discuss the within-group comparisons.

Placebo. Following placebo reversal and recovery of the train-of-four ratio to values more than 0.90 as measured at the thumb, the hypoxic response was just 76% of control, whereas ventilation at an extrapolated ET_{CO_2} of 55 mmHg was fully restored. The resultant reduced carotid body index indicates that despite the return of muscle function at the thumb, carotid body function remained suboptimal. A possible explanation is a difference in rocuronium affinity for muscle *versus* neuronal nicotinic acetylcholine receptors. *In vitro* experiments give evidence for the distinct pharmacologic properties of rocuronium at various human muscle and neuronal nicotinic acetylcholine receptor subtypes expressed on *Xenopus* oocytes(21). However, functional affinity of nondepolarizing muscle relaxants was higher for muscle type receptors, with half-maximum inhibitory concentrations in the nanomolar concentration range for muscle nicotinic acetylcholine receptors *versus* micromolar range for neuronal nicotinic acetylcholine receptors. This seems to contradict our findings. Still, reversal of rocuronium-impaired carotid body function is a dynamic process that is influenced by several factors, such as acetylcholine receptors receptor kinetics at pre- and postsynaptic sites (including involvement of specific receptor subtypes), local blood flow, local acetylcholine and acetylcholinesterase concentrations, interaction with other (including muscarinic acetylcholine, dopamine, purinergic) receptors, receptor (de)sensitization, neuronal dynamics, *etc*. Currently, little is known about this complex process, and we postulate that reversal of the rocuronium effect at carotid bodies is slower than at peripheral muscles. This might not be so for the other non-depolarizing neuromuscular blockers, because Eriksson(8) showed full recovery of hypoxic responses and F values following spontaneous return of the train-of-four ratio to values of more than 0.9 after partial muscle relaxation induced by vecuronium, atracurium, and pancuronium.(8)

Neostigmine. Seven subjects (64%) had corrected F3 values less than 0.95 following reversal. Consequently, we argue that although neostigmine was effective in restoring muscle function at the thumb, it did not concurrently restore the hypoxic response in all subjects. We cannot exclude a possible role for atropine in this suboptimal reversal. Animal data indicate that atropine, a muscarinic acetylcholine receptor antagonist, significantly attenuates the carotid body response to hypoxia.(22,23) Because data derived from human carotid bodies detected expression of just neuronal nicotinic acetylcholine receptors(24), a possible inhibitory effect of atropine on the hypoxic response in our sample seems unlikely. What remains is the possibility that the neostigmine dose (13 µg · kg−1) was sufficient to reverse respiratory muscle impairment but insufficient for full reversal of carotid body function in some subjects. Dose-response studies are needed to determine the dose required to restore carotid body function in all individuals following rocuronium relaxation.

Sugammadex. Five subjects (45%) had corrects F3 values less than 0.95. Consequently, even with sugammadex reversal and restoration of muscle function at the thumb, the hypoxic response may not be restored in all patients.

Our data indicate that reversal to a train-of-four ratio of 1 does not fully reverse blunting of the acute hypoxic response in most subjects. Because our protocol was unable to determine the timing at which the response returned to baseline values (*i.e.*, AHR3 > 0.95 × AHR1), further studies are urgently needed to establish the dynamics of the return of the hypoxic response toward baseline following reversal strategies (neostigmine/atropine, sugammadex) or spontaneous recovery.

Trial Limitations

Although we randomized the hypoxic and hypercapnic tests during relaxation, we refrained from randomization following reversal. Although this was done to ensure that the hypoxic tests were performed at similar stages of reversal, this may have affected the hypercapnic test results. Indeed, ventilation at an extrapolated ET_{CO_2} of 55 mmHg values following reversal were on average 15% larger than control values. This observation made us decide to correct for this excitatory effect by constraining the upper limit of VE553R to 100% to prevent overestimation of the effect of the three reversal agents. Uncorrected and corrected F3 values differed by about 10%. However, because 22 of 34 subjects had a corrected F3 value of less than 0.95, we do not believe that this correction affected the clinical interpretation of the data.

Our method of measuring muscle relaxation may have been suboptimal. Although mechanomyography is considered the gold standard in neuromuscular monitoring, electromyography is generally regarded a good and accurate alternative to mechanomyography(25) and lacks the staircase effect that troubles acceleromyography or mechanomyography(26). However, measurements may have been influenced by the fact that our subjects were awake, which may have caused occasional (unnoticed) thumb movements disturbing the measurements with possibly some overestimation of the train-of-four ratio. In addition, supramaximal stimulation was not used to avoid a possible confounding effect of discomfort on respiratory measurements.

Finally, train-of-four ratios were not corrected for baseline train-of-four ratio, which may have caused overestimation of the recovery of the neuromuscular block at the time of measurement.

However, this effect was relatively small (1 to 2%).

In conclusion, we successfully replicated the original study by Eriksson *et al.*(6) showing an inhibitory effect of rocuronium at the carotid bodies. Our reversal data point toward persistence of carotid body impairment despite recovery of the train-of-four ratio to values of more than 0.9 at the thumb. These are important and clinically relevant observations. However, given the complexity of this experimental study, we highlight the need for further investigations. We encourage others to replicate our study and address the effect of spontaneous return of neuromuscular function following rocuronium relaxation on carotid body function. Finally, we would like to stress that in clinical practice, residual anesthetic and muscle relaxant levels synergistically affect breathing postoperatively. Further research should investigate such interactions with special emphasis on mechanisms and sites of action.

References

1. Sundman E, Witt H, Olsson R, Ekberg O, Kuylenstierna R, Eriksson LI: The incidence and mechanisms of pharyngeal and upper esophageal dysfunction in partially paralyzed humans: Pharyngeal videoradiography and simultaneous manometry after atracurium. Anesthesiology 2000; 92:977–84
2. Eikermann M, Groeben H, Husing J, Peters J: Accelerometry of adductor pollicis muscle predicts recovery of respiratory function from neuromuscular blockade. Anesthesiology 2003; 98:1333–7
3. Eikermann M, Vogt FM, Herbstreit F, Vahid-Dastgerdi M, Zenge MO, Ochterbeck C, de Greiff A, Peters J: The predisposition to inspiratory upper airway collapse during partial neuromuscular blockade. Am J Respir Crit Care Med 2007; 175:9–15
4. Murphy GS, Brull SJ: Residual neuromuscular block: Lessons unlearned: Part I. Definitions, incidence, and adverse physiologic effects of residual neuromuscular block. Anesth Analg 2010; 111:120–8
5. Boon M, Martini C, Broens S, van Rijnsoever E, van der Zwan T, Aarts L, Dahan A: Improved postoperative oxygenation after antagonism of moderate neuromuscular block with sugammadex *versus* neostigmine after extubation in "blinded" conditions. Br J Anaesth 2016; 117:410–1
6. Eriksson LI, Lennmarken C, Wyon N, Johnson A: Attenuated ventilatory response to hypoxaemia at vecuronium-induced partial neuromuscular block. Acta Anaesthesiol Scand 1992; 36:710–5
7. Eriksson LI, Sato M, Severinghaus JW: Effect of a vecuronium-induced partial neuromuscular block on hypoxic ventilatory response. Anesthesiology 1993; 78:693–9
8. Eriksson LI: Reduced hypoxic chemosensitivity in partially paralysed man: A new property of muscle relaxants? Acta Anaesthesiol Scand 1996; 40:520–3
9. Jonsson M, Wyon N, Lindahl SG, Fredholm BB, Eriksson LI: Neuromuscular blocking agents block carotid body neuronal nicotinic acetylcholine receptors. Eur J Pharmacol 2004; 497:173–80
10. Igarashi A, Amagasa S, Horikawa H, Shirahata M: Vecuronium directly inhibits hypoxic neurotransmission of the rat carotid body. Anesth Analg 2002; 94:117–22
11. O'Donohoe PB, Turner PJ, Huskens N, Buckler KJ, Pandit JJ: Influence of propofol on isolated neonatal rat carotid body glomus cell response to hypoxia and hypercapnia. Respir Physiol Neurobiol 2019; 260:17–27
12. Teppema LJ, Dahan A: The ventilatory response to hypoxia in mammals: Mechanisms, measurement, and analysis. Physiol Rev 2010; 90:675–754
13. Berkenbosch A, Teppema LJ, Olievier CN, Dahan A: Influences of morphine on the ventilatory response to isocapnic hypoxia. Anesthesiology 1997; 86:1342–9
14. Dahan A, Teppema LJ: Influence of anaesthesia and analgesia on the control of breathing. Br J Anaesth 2003;91:40–9
15. Dahan A, DeGoede J, Berkenbosch A, Olievier IC: The influence of oxygen on the ventilatory response to carbon dioxide in man. J Physiol 1990; 428:485–99

16. Dahan A, Nieuwenhuijs D, Teppema L: Plasticity of central chemoreceptors: Effect of bilateral carotid body resection on central CO2 sensitivity. PLoS Med 2007;4:e239
17. van der Schrier R, Roozekrans M, Olofsen E, Aarts L, van Velzen M, de Jong M, Dahan A, Niesters M: Influence of ethanol on oxycodone-induced respiratory depression: A dose-escalating study in young and elderly volunteers. Anesthesiology 2017; 126:534–42
18. Kleijn HJ, Zollinger DP, van den Heuvel MW, Kerbusch T: Population pharmacokinetic–pharmacodynamics analysis for sugammadex-mediated reversal of rocuronium-induced neuromuscular blockade. Br J Clin Pharmacol 2011; 72:415–33
19. Hodgson PS, Liu SS, Gras TW: Does epidural anesthesia have general anesthetic effects? A prospective, randomized, double-blind, placebo-controlled trial. Anesthesiology 1999; 91:1687–92
20. Niesters M, Sitsen E, Oudejans L, Vuyk J, Aarts LP, Rombouts SA, de Rover M, Khalili-Mahani N, Dahan A: Effect of deafferentation from spinal anesthesia on pain sensitivity and resting-state functional brain connectivity in healthy male volunteers. Brain Connect 2014; 4:404–16
21. Jonsson M, Gurley D, Dabrowski M, Larsson O, Johnson EC, Eriksson LI: Distinct pharmacologic properties of neuromuscular blocking agents on human neuronal nicotinic acetylcholine receptors: A possible explanation for the train-of-four fade. Anesthesiology 2006; 105:521–33
22. Kumar P, Prabhakar NR: Peripheral chemoreceptors: Function and plasticity of the carotid body. Compr Physiol 2012; 2:141–219
23. Dasso LL, Buckler KJ, Vaughan-Jones RD: Muscarinic and nicotinic receptors raise intracellular Ca2+ levels in rat carotid body type I cells. J Physiol 1997; 498:327–38
24. Fagerlund MJ, Kahlin J, Ebberyd A, Schulte G, Mkrtchian S, Eriksson LI: The human carotid body: Expression of oxygen sensing and signaling genes of relevance for anesthesia. Anesthesiology 2010; 113:1270–9
25. Engbaek J, Roed J, Hangaard N, Viby-Mogensen J: The agreement between adductor pollicis mechanomyogram and first dorsal interosseous electromyogram: A pharmacodynamic study of rocuronium and vecuronium. Acta Anaesthesiol Scand 1994; 38:869–78
26. Kopman AF, Kumar S, Klewicka MM, Neuman GG: The staircase phenomenon: Implications for monitoring of neuromuscular