

# Neuromuscular Blockade with Rocuronium Bromide Increases the Tolerance of Acute Normovolemic Anemia in Anesthetized Pigs

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## Key Words

Neuromuscular blockade · Rocuronium bromide · Anemia tolerance · Pig

## Abstract

**Background:** The patient's individual anemia tolerance is pivotal when blood transfusions become necessary, but are not feasible for some reason. To date, the effects of neuromuscular blockade (NMB) on anemia tolerance have not been investigated. **Methods:** 14 anesthetized and mechanically ventilated pigs were randomly assigned to the Roc group (3.78 mg/kg rocuronium bromide followed by continuous infusion of 1 mg/kg/min, n = 7) or to the Sal group (administration of the corresponding volume of normal saline, n = 7). Subsequently, acute normovolemic anemia was induced by simultaneous exchange of whole blood for a 6% hydroxyethyl starch solution (130/0.4) until a sudden decrease of total body O<sub>2</sub> consumption (VO<sub>2</sub>) indicated a critical limitation of O<sub>2</sub> transport capacity. The Hb concentration quantified at this time point (Hb<sub>crit</sub>) was the primary endpoint of the protocol. Secondary endpoints were parameters of hemodynamics, O<sub>2</sub> transport and tissue oxygenation. **Results:** Hb<sub>crit</sub> was significantly lower in the Roc group (2.4 ± 0.5 vs. 3.2 ± 0.7 g/dl) reflecting increased anemia tolerance.

NMB with rocuronium bromide reduced skeletal muscular VO<sub>2</sub> and total body O<sub>2</sub> extraction rate. As the cardiac index increased simultaneously, total body VO<sub>2</sub> only decreased marginally in the Roc group (change of VO<sub>2</sub> relative to baseline -1.7 ± 0.8 vs. 3.2 ± 1.9% in the Sal group, p < 0.05). **Conclusion:** Deep NMB with rocuronium bromide increases the tolerance of acute normovolemic anemia. The underlying mechanism most likely involves a reduction of skeletal muscular VO<sub>2</sub>. During acellular treatment of an acute blood loss, NMB might play an adjuvant role in situations where profound stages of normovolemic anemia have to be tolerated (e.g. bridging an unexpected blood loss until blood products become available for transfusion).

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## Introduction

The initial treatment of an acute blood loss usually consists of the infusion of crystalloid and/or colloidal solutions. The goal is the maintenance of normovolemia, the result is a dilution of the red cell mass remaining in the vasculature (acute normovolemic anemia). The term 'anemia tolerance' refers to both the patient's physiological ability to tolerate even profound stages of acute ane-

mia as well as to the anesthesiologist's willingness to accept low Hb concentrations [1].

Owing to the organism's individual anemia tolerance, tissue oxygenation is maintained, although O<sub>2</sub> transport capacity decreases progressively with dilutional anemia. Acute anemia is initially compensated by increases in cardiac output and arteriovenous O<sub>2</sub> extraction [2]. At physiologic Hb concentrations, O<sub>2</sub> supply to the tissues (DO<sub>2</sub>) exceeds total body O<sub>2</sub> demand – reflected by O<sub>2</sub> consumption (VO<sub>2</sub>) under rest conditions – by the factor 3–4 [3]. Even when DO<sub>2</sub> begins to decrease at lower Hb concentrations, O<sub>2</sub> supply to the tissues is still sufficient to meet their O<sub>2</sub> demand and VO<sub>2</sub> remains constant over a wide range of decreasing Hb concentrations (supply independency of VO<sub>2</sub>). When DO<sub>2</sub> falls below a critical value, the amount of O<sub>2</sub> delivered to the tissues becomes insufficient to meet their O<sub>2</sub> demand and VO<sub>2</sub> starts to decline (supply dependency of VO<sub>2</sub> [4, 5]). Hb<sub>crit</sub> is the Hb concentration corresponding to the sudden decrease in VO<sub>2</sub> and reflects the individual limit of anemia tolerance [6].

Neuromuscular blockade (NMB) is an integral part of the anesthetic management of a variety of surgical interventions. While NMB has been demonstrated to decrease total body energy expenditure and VO<sub>2</sub> [7, 8], to date, a potential influence on anemia tolerance has not been investigated. We hypothesized that NMB should increase the tolerance of acute normovolemic anemia – reflected by a significantly lower value of Hb<sub>crit</sub> and a higher volume of blood allowed to be exchanged for hydroxyethyl starch (HES).

## Materials and Methods

After approval by the local governmental review board, experiments were performed in 20 healthy farm-bred pigs of both sexes (body weight 25.8 ± 3.7 kg). All animals received good care in compliance with the Guide for the Care and Use of Laboratory Animals. Prior to the actual study, 5 pilot experiments were performed for dose finding (identification of an adequate dose of rocuronium bromide) and to assess potential effects of NMB on skeletal muscular VO<sub>2</sub> (mVO<sub>2</sub>).

### *Anesthesia and Ventilation*

Twelve hours before the experiments, the animals were denied food but had free access to water. After intramuscular premedication with 10 mg/kg ketamine and 1 mg/kg midazolam, anesthesia was induced by intravenous injection of 3 mg/kg propofol and 30 µg/kg fentanyl. Anesthesia was maintained by continuous infusion of propofol (0.16 mg/kg/min), midazolam (0.01 mg/kg/min) and fentanyl (0.8 µg/kg/min). Estimated fluid losses were replaced with a balanced electrolyte solution (3 ml/kg/h). All trade

names of anesthetics and infusion fluids applied are listed in the Appendix.

The animals' tracheas were orally intubated and their lungs were ventilated with ambient air at a rate of 14 cycles/min and a positive end-expiratory pressure of 5 cm H<sub>2</sub>O (Servo 900B, Siemens Elema, Solna, Sweden). Tidal volume was individually adjusted to provide arterial normocapnia and was then maintained throughout the entire procedure. Body core temperature was kept constant using a warming pad.

### *Pilot Experiments*

For preliminary assessment of mVO<sub>2</sub>, the right femoral artery and vein were dissected free from surrounding tissue. An ultrasonic flow probe (diameter 2 mm, Transsonic, Ithaca, N.Y., USA) was placed around the femoral artery and the femoral vein was cannulated with an 18-gauge Teflon catheter (Leader Cath, Vygon, Ecouen, France), which was inserted retrograde for 15 mm. An area of 3 × 5 cm of the adductor muscle of the lower limb was dissected free from surrounding tissue for measurement of tissue O<sub>2</sub> partial pressure. Thirty minutes after completion of surgical preparation, baseline values of mVO<sub>2</sub> and tissue O<sub>2</sub> partial pressure were obtained. Subsequently, the dissected skeletal muscle was subjected to tetanic electrical stimulation using a relaxometer device (TOF-watch, Organon, Oberschleissheim, Germany) and the second data set was recorded ('stimulation'). After a 30-min recovery period, a complete NMB was established with 3.78 mg/kg of rocuronium bromide ('relaxation'). In pigs, the ED<sub>95</sub> of rocuronium was determined to be 1.26 mg/kg [9], so that the used dose corresponded with the threefold ED<sub>95</sub>. The final data set was obtained 2 min after reversal of NMB with the tenfold dose (37.8 mg/kg) of sugammadex ('reversal'). Subsequently, the animals were killed by intracardial injection of saturated potassium chloride solution.

### *Study Experiments*

#### *Instrumentation and Monitoring*

The animals were placed in supine position and a 5-lead electrocardiogram (II, V5) was used for detection of arrhythmias and ST segment changes. A double-lumen catheter (Arrow, Reading, Pa., USA) was inserted into the cranial vena cava and a Swan-Ganz catheter (Baxter, Irvine, Calif., USA) was floated into a branch of the pulmonary artery; 6-french introducer sheaths were inserted into both the right femoral artery and vein, respectively. For continuous measurement of arterial blood pressure and cardiac output, a thermodilution catheter (Pulsion, Munich, Germany) was placed into the left femoral artery.

#### *Experimental Protocol*

Upon placement of the different measuring devices, a 60-min stabilization period was allowed to elapse to achieve stable baseline conditions. The first data set ('baseline') was recorded, and appropriate neuromuscular transmission was visualized by assessment of the right foreleg extensor muscle contraction response to electric train-of-four stimulation (TOF-watch).

Thereafter, the animals were randomized to receive either rocuronium bromide or normal saline (Roc group, n = 7 vs. Sal group, n = 7). In the Roc group, the threefold ED<sub>95</sub> (3.78 mg/kg) was administered, followed by continuous infusion of 1 mg/kg/min. In the Sal group, the animals received the corresponding volumes of normal saline. The investigator remained blinded un-

til the administration of rocuronium or saline was terminated and the second data set ('treatment') was registered.

Subsequently, an automated hemodilution protocol was initiated by isovolumic exchange of whole blood for HES (6% HES 130/0.4, 1 ml/kg/min). The target parameter was the animal's individual  $Hb_{crit}$  concentration, which was prospectively assessed in an investigator-independent manner (see below).

In the Roc group, the completeness of NMB was confirmed every 60 s during the entire hemodilution protocol (TOF stimulation of the foreleg extensor muscle). Therefore, the investigator needed to be unblinded at the initiation of the hemodilution protocol. When  $Hb_{crit}$  was met, the final data set was collected (' $Hb_{crit}$ ') and the animals were killed by intracardial injection of saturated potassium chloride solution.

#### Assessment of $Hb_{crit}$

$Hb_{crit}$  is the correlate of the critical limitation of  $DO_2$ , and marks the onset of total body  $O_2$  supply dependency. The corresponding decrease in  $VO_2$  was detected in an automated and investigator-independent manner:  $VO_2$  was measured every 60 s with a metabolic monitor (Delta-Trac II™ MBM-200, Datex-Engstrom, Helsinki, Finland).  $VO_2$  values were simultaneously recorded and computed with a specific software (DeltaCrit System) [10]. During the stabilization period, the DeltaCrit System included  $VO_2$  values into an online regression analysis and calculated mean and standard deviation. During the subsequent hemodilution period, every minutely obtained  $VO_2$  value was compared to the mean value predicted by the DeltaCrit System. When three consecutive  $VO_2$  values were outside the predefined range ( $3 \times$  standard deviation of regression line; fig. 1), a significant decrease in  $VO_2$  was assumed and signaled by visual and acoustic computer alerts [10].

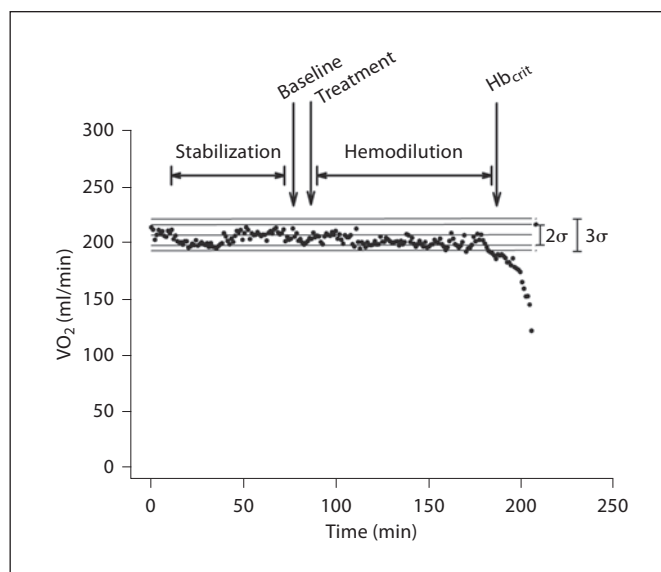
#### Measurements

Measurements of hemodynamic and  $O_2$ -derived parameters were performed at 'baseline', 'treatment' and at ' $Hb_{crit}$ '. Intravascular blood volume was determined at baseline using the 'whole-blood' method of the indocyanine green indicator dilution technique, which has already been described in detail elsewhere [11]. The pressure transducers of the cardiovascular catheters were connected with a multichannel recorder (Hugo-Sachs, March-Hugstetten, Germany) and measurement readings were recorded with a personal computer. Cardiac output was continuously assessed using the transpulmonary thermodilution technique (PICCO-Classic monitor, Pulsion, Munich, Germany). Arterial and mixed venous blood samples were withdrawn for blood gas analysis and assessment of Hb concentration (GEM 3000 and 682 CO-Oximeter, both Instrumentation Laboratory, Lexington, Mass., USA). Calculated parameters were determined as described in the Appendix.

#### Statistics

Statistical analysis was performed with the SAS 9.1 software package (SAS Institute, Cary, N.C., USA). Distribution of data was assessed with the Shapiro-Wilk test. Normally distributed parameters are presented as mean  $\pm$  standard deviation, nonnormally distributed parameters are displayed as median  $\pm$  semi-interquartile range.

In the case of normal distribution, the time effect on the different variables as well as differences between the groups at the



**Fig. 1.** Typical example of the continuous  $VO_2$  recording and identification of  $Hb_{crit}$ .  $VO_2$  values collected during the 60 min were included in a linear regression analysis and mean and standard deviation were calculated. During the hemodilution protocol, every current  $VO_2$  value was compared with the mean value determined during the stabilization period. A critical limitation of  $DO_2$  was assumed, when three consecutive  $VO_2$  values fell below the lower  $3\sigma$  range.

investigated time points were tested by repeated analysis of variance. Post hoc analysis of differences detected with analysis of variance was performed with the Student-Newman-Keuls test.

In the case of nonnormal distribution, the time effect on the parameters as well as between-group differences were tested by analysis of variance on ranks. Post hoc analysis of differences detected with analysis of variance on ranks was performed with Tukey's test. For all parameters, statistical significance was accepted at  $p < 0.05$ .

The sample sizes were calculated on the basis of a postulated power of 85% and a variance of data observed in our previous studies with a similar experimental protocol.

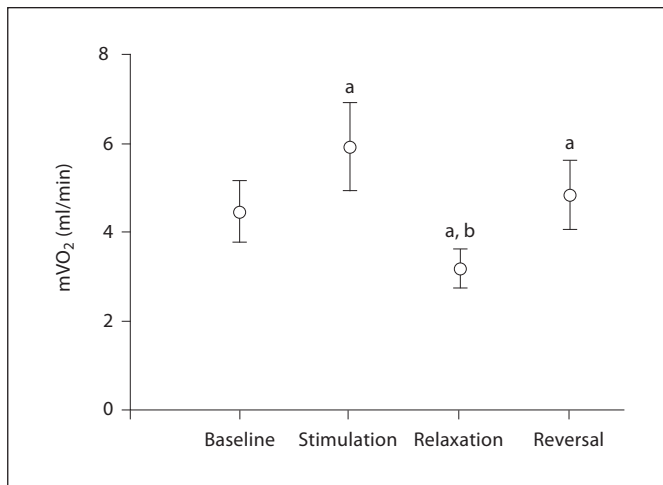
## Results

### Pilot Experiments

Following neurostimulation of the lower limb adductor muscle, skeletal  $mVO_2$  was significantly increased (fig. 2).

The increase in  $mVO_2$  was accompanied by an increase in femoral arterial blood flow, while muscular  $O_2$  extraction rate did not change significantly (table 1).

After a 30-min recovery period, NMB with rocuronium bromide decreased  $mVO_2$  beyond the baseline level



**Fig. 2.** Skeletal mVO<sub>2</sub> determined in the lower limb adductor muscle (for the calculation of mVO<sub>2</sub>, see Appendix). mVO<sub>2</sub> was assessed in the pilot study at the following time points: (1) after termination of surgical preparation ('baseline'), (2) after electrical neurostimulation of the muscle ('stimulation'), (3) after a 30-min recovery period and subsequent implementation of NMB ('relaxation'), and (4) after reversal of NMB with sugammadex ('reversal'). Neurostimulation was associated with a significant increase in mVO<sub>2</sub>. NMB decreased mVO<sub>2</sub> beyond the baseline level, while the reversal of NMB returned mVO<sub>2</sub> to baseline. <sup>a</sup> p < 0.05 vs. previous time point, <sup>b</sup> p < 0.05 vs. baseline.

(p = 0.011; fig. 1), which was accompanied by a significant decrease in femoral arterial blood flow and a nonsignificant decrease in muscular O<sub>2</sub> extraction rate (table 1). After pharmacologic reversal of NMB, mVO<sub>2</sub> and femoral arterial blood flow returned to baseline, which was paralleled by a significant increase in muscular O<sub>2</sub> extraction rate (table 1).

Contrasting the effects on mVO<sub>2</sub>, VO<sub>2</sub> remained unchanged after neurostimulation, NMB, and after the administration of sugammadex.

### Study Experiments

#### Baseline Characteristics

No significant differences between the groups were observed at baseline (table 2).

#### Effects of Treatment

One animal developed a fatal allergic reaction immediately after injection of rocuronium bromide; this experiment was not included in data analysis as the animal did not meet the inclusion criterion (completion of hemodilution protocol).

**Table 1.** Pilot experiments: parameters of global and regional O<sub>2</sub> transport

Parameter	Baseline	Stimulation	Relaxation	Reversal
HR, min <sup>-1</sup>	70 ± 4	67 ± 5	76 ± 8	74 ± 4
MAP, mm Hg	79 ± 13	81 ± 5	85 ± 13	77 ± 10
VO <sub>2</sub> I, ml·min <sup>-1</sup> ·m <sup>-2</sup>	237 ± 13	241 ± 19	236 ± 20	233 ± 16
Q, ml·min <sup>-1</sup>	89 ± 8	136 ± 5*	84 ± 8*	98 ± 10*
SaO <sub>2</sub> , %	94 ± 1	94 ± 2	94 ± 2	94 ± 2
paO <sub>2</sub> , mm Hg	77 ± 8	76 ± 10	76 ± 13	78 ± 10
SfvO <sub>2</sub> , %	62 ± 9	63 ± 6	66 ± 2	57 ± 4*
pfvO <sub>2</sub> , mm Hg	33 ± 3	36 ± 3	35 ± 3	32 ± 2*
tpO <sub>2</sub> , mm Hg	30 ± 9	39 ± 23	39 ± 11	34 ± 24
mO <sub>2</sub> -ER, %	35 ± 9	33 ± 6	29 ± 4	39 ± 5*

HR = Heart rate; MAP = mean arterial pressure; VO<sub>2</sub>I = total body O<sub>2</sub> consumption indexed to body surface area; Q = femoral arterial blood flow; SaO<sub>2</sub>, paO<sub>2</sub>, SfvO<sub>2</sub>, pfvO<sub>2</sub> = arterial and femoral venous O<sub>2</sub> saturation and O<sub>2</sub> partial pressure; tpO<sub>2</sub> = tissue O<sub>2</sub> partial pressure; mO<sub>2</sub> ER = muscular O<sub>2</sub> extraction rate. \* p < 0.05 vs. previous.

While the injection of normal saline was devoid of any hemodynamic effect, the administration of rocuronium bromide was associated with significant increases in heart rate and mean pulmonary artery pressure and nonsignificant increases in cardiac index (CI) and pulmonary capillary wedge pressure. Compared with the Sal group, the mean pulmonary artery pressure was higher and the systemic vascular resistance index was lower in animals having received rocuronium bromide (p < 0.05; table 2).

Although the O<sub>2</sub> extraction rate was decreased in the Roc group (p < 0.05 vs. baseline and vs. Sal), NMB did not exert a significant change of total body VO<sub>2</sub> (fig. 3). However, in 5 of the 7 animals of the Roc group, VO<sub>2</sub> decreased slightly, while VO<sub>2</sub> only decreased in 2 animals of the Sal group. As a consequence, the change of VO<sub>2</sub> relative to baseline (ΔVO<sub>2</sub>) had a negative sign in the Roc group (ΔVO<sub>2</sub>: -1.7 ± 0.8%) and was positive in the Sal group (3.2 ± 1.9%; fig. 2).

#### Primary Endpoint: Hb<sub>crit</sub>

NMB with rocuronium bromide enabled a more extensive blood for HES exchange: in the Roc group, 2,081 ± 449 ml of blood had been exchanged (vs. 1,433 ± 501 ml in the Sal group, p = 0.014) until Hb<sub>crit</sub> was met and the hemodilution protocol was terminated. As a consequence, a significantly lower value of Hb<sub>crit</sub> was attained in the Roc group (2.4 ± 0.5 g/dl vs. 3.2 ± 0.7 g/dl, p = 0.015; fig. 4).

**Table 2.** Parameters of central hemodynamics and O<sub>2</sub> transport obtained at baseline, after treatment with Roc or Sal, and at the Hb<sub>crit</sub>

Parameter	Group	Baseline	Treatment	Hb <sub>crit</sub>
BVI ml·m <sup>-2</sup>	Roc	81 ± 13	n.d.	n.d.
	Sal	72 ± 7	n.d.	n.d.
Temperature °C	Roc	37.5 ± 1.1	37.4 ± 1.4	37.1 ± 1.0
	Sal	37.3 ± 0.8	37.2 ± 0.5	37.0 ± 0.8
HR min <sup>-1</sup>	Roc	77 ± 7	93 ± 12 <sup>a</sup>	125 ± 11 <sup>a</sup>
	Sal	79 ± 7	83 ± 7	117 ± 19 <sup>a</sup>
MAP mm Hg	Roc	73 ± 10	76 ± 12	49 ± 11 <sup>a</sup>
	Sal	86 ± 19	82 ± 10	73 ± 8 <sup>a, b</sup>
MPAP mm Hg	Roc	24 ± 4	30 ± 5 <sup>a, b</sup>	28 ± 1
	Sal	23 ± 7	24 ± 7	29 ± 7 <sup>a</sup>
PCWP mm Hg	Roc	5.3 ± 2.2	8.2 ± 3.2	7.4 ± 3.3
	Sal	5.0 ± 2.9	5.6 ± 3.8	7.7 ± 4.7 <sup>a</sup>
CI l·min <sup>-1</sup> ·m <sup>2</sup>	Roc	3.9 ± 0.6	4.6 ± 0.9	7.3 ± 1.1 <sup>a</sup>
	Sal	3.5 ± 0.2	3.9 ± 0.4	7.2 ± 1.1 <sup>a</sup>
SVI ml·m <sup>-2</sup>	Roc	53 ± 4	49 ± 4	57 ± 9
	Sal	45 ± 4	47 ± 6	59 ± 4 <sup>a</sup>
CVP mm Hg	Roc	5.5 ± 4.1	5.8 ± 2.8	7.6 ± 2.0
	Sal	6.5 ± 3.0	5.6 ± 2.9	8.9 ± 4.1
SVR dyn <sup>-1</sup> ·s <sup>-1</sup> ·cm <sup>-5</sup>	Roc	1,795 ± 241	1,319 ± 388	627 ± 474 <sup>a</sup>
	Sal	1,660 ± 183	1,554 ± 134 <sup>b</sup>	742 ± 119 <sup>a</sup>
PVRI dyn <sup>-1</sup> ·s <sup>-1</sup> ·cm <sup>-5</sup>	Roc	383 ± 48	431 ± 91	248 ± 43
	Sal	421 ± 116	378 ± 105	218 ± 26 <sup>a</sup>
CPP mm Hg	Roc	51 ± 10	53 ± 12	25 ± 5 <sup>a</sup>
	Sal	64 ± 18	58 ± 8	45 ± 8 <sup>a, b</sup>
VO <sub>2</sub> I ml <sup>-1</sup> ·min <sup>-1</sup> ·m <sup>-2</sup>	Roc	231 ± 20	228 ± 22	199 ± 28 <sup>a</sup>
	Sal	241 ± 18	244 ± 14	226 ± 16 <sup>a</sup>
DO <sub>2</sub> I ml <sup>-1</sup> ·min <sup>-1</sup> ·m <sup>-2</sup>	Roc	425 ± 78	496 ± 74	227 ± 94 <sup>a, b</sup>
	Sal	374 ± 8	429 ± 62	327 ± 55 <sup>a</sup>
paO <sub>2</sub> mm Hg	Roc	99 ± 13	96 ± 14	95 ± 8
	Sal	105 ± 12	110 ± 8	113 ± 23
paCO <sub>2</sub> mm Hg	Roc	34 ± 4	35 ± 4	35 ± 2
	Sal	33 ± 3	33 ± 3	34 ± 3
CaO <sub>2</sub> ml·dl <sup>-1</sup>	Roc	10.8 ± 1.1	11.1 ± 1.0	3.5 ± 0.7 <sup>a</sup>
	Sal	10.8 ± 0.6	10.9 ± 0.9	4.6 ± 1.0 <sup>a, b</sup>
SvO <sub>2</sub> %	Roc	57 ± 10	62 ± 8 <sup>a</sup>	53 ± 12 <sup>a</sup>
	Sal	53 ± 8	56 ± 9	61 ± 18
O <sub>2</sub> -ER %	Roc	43 ± 10	34 ± 6 <sup>a, b</sup>	50 ± 11 <sup>a</sup>
	Sal	47 ± 7	41 ± 8	41 ± 16
pH	Roc	7.55 ± 0.08	7.49 ± 0.11	7.47 ± 0.03 <sup>a, b</sup>
	Sal	7.57 ± 0.04	7.58 ± 0.04	7.52 ± 0.04 <sup>a</sup>
Base excess mmol·l <sup>-1</sup>	Roc	7.5 ± 4.0	3.5 ± 5.5	1.4 ± 2.4 <sup>a, b</sup>
	Sal	8.4 ± 2.4	8.3 ± 2.0	4.6 ± 1.8 <sup>a</sup>
Lactate mmol·l <sup>-1</sup>	Roc	1.7 ± 1.0	1.7 ± 1.0	2.1 ± 1.0
	Sal	2.1 ± 0.6	2.0 ± 0.6	2.0 ± 0.8

BVI = Blood volume indexed to body surface area; HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; SVI = stroke volume index; CVP = central venous pressure; SVRI and PVRI = systemic and pulmonary vascular resistance index; CPP = coronary perfusion pressure; DO<sub>2</sub>I = O<sub>2</sub> delivery indexed to body surface area; paO<sub>2</sub> = arterial O<sub>2</sub> partial pressure; CaO<sub>2</sub> = arterial O<sub>2</sub> content; SvO<sub>2</sub> = mixed venous O<sub>2</sub> saturation; O<sub>2</sub> ER = O<sub>2</sub> extraction rate. Roc group, n = 7; Sal group, n = 7. <sup>a</sup> p < 0.05 difference vs. baseline; <sup>b</sup> p < 0.05 difference between the groups.

## Secondary Endpoints: Hemodynamics and O<sub>2</sub> Transport

During induction of critical normovolemic anemia, heart rate and CI increased in both groups by 38 and 67% (p < 0.05 vs. baseline), whereas mean aortic pressure and coronary perfusion pressure decreased by 23 and 37%, respectively (p < 0.05 vs. baseline). PVRI decreased by 10% in the Roc group (nonsignificant vs. baseline) and by 38% in the Sal group (p < 0.05 vs. baseline). Furthermore, hemodilution was associated with a decrease of CaO<sub>2</sub>, DO<sub>2</sub>, pH and BE by 64, 59, 1 and 49% (p < 0.05 vs. baseline). The O<sub>2</sub> extraction rate only increased in the Roc group (p < 0.05), which was accompanied by decreases in SvO<sub>2</sub> and pvO<sub>2</sub> (p < 0.05).

At Hb<sub>crit</sub>, the following differences between the groups were observed: arterial blood pressure was significantly lower in the Roc group, resulting in lower values of mean aortic pressure and coronary perfusion pressure (p < 0.05). Due to the lower Hb<sub>crit</sub> in the Roc group, CaO<sub>2</sub> and DO<sub>2</sub> indexed to the body surface area were significantly lower than in the Sal group. The lower values of pH and base excess (p < 0.05) reflect dilutional acidosis related to a more profound degree of hemodilution in the Roc group.

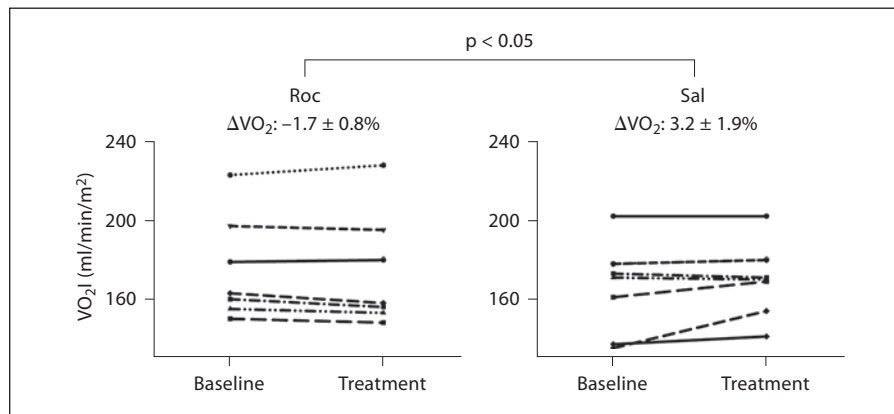
## Discussion

The main result of the present study is that NMB with rocuronium bromide increased the tolerance of acute normovolemic anemia, as indicated by a significantly lower value of Hb<sub>crit</sub> and a higher volume of blood exchanged for HES until Hb<sub>crit</sub> was met.

Hb<sub>crit</sub> represents the ultimate limit of anemia tolerance, i.e., the Hb concentration corresponding with a critical limitation of DO<sub>2</sub>. When Hb falls short of this critical value, the amount of O<sub>2</sub> delivered to the tissues becomes insufficient to meet their O<sub>2</sub> demand and VO<sub>2</sub> starts to decline (onset of O<sub>2</sub> supply dependency of VO<sub>2</sub>) [4, 5]. In our previous experimental studies, critical normovolemic anemia was associated with 100% mortality if no further treatment (e.g. elevation of FiO<sub>2</sub>, transfusion of red blood cells, administration of catecholamines, infusion of artificial O<sub>2</sub> carriers) was instantaneously initiated at Hb<sub>crit</sub> [12–15].

Hb<sub>crit</sub> has been chosen as the primary endpoint in several experimental studies investigating the impact of several interventions on the tolerance limit of acute anemia and was usually found at values between 1.6 and 3 g/dl (table 3). Consistently, the value of Hb<sub>crit</sub> in the Roc group

**Fig. 3.** Change of  $VO_2$  after treatment displayed as mean difference relative to baseline ( $\Delta VO_2$ ). In 5 out of 7 animals of the Roc group,  $VO_2$  decreased slightly ( $\Delta VO_2 -1.7 \pm 0.8\%$ ), while in the Sal group,  $VO_2$  only decreased in 2 out of 7 animals ( $\Delta VO_2 3.2 \pm 1.9\%$ ,  $p < 0.05$  vs. Roc group).



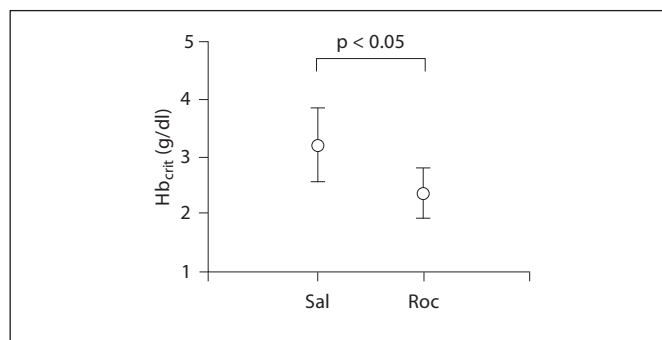
( $2.4 \pm 0.5$  g/dl) is comparable to that described in paralyzed animals [13, 15–19], while the values of  $Hb_{crit}$  obtained in nonparalyzed animals are similar to  $Hb_{crit}$  observed in the Sal group [4, 12].

In several experimental studies, a decrease in  $Hb_{crit}$  demonstrated that anemia tolerance is increased by (1) hypothermia (moderate reduction of body core temperature reduces total body  $O_2$  demand [16]), (2) hyperoxic ventilation (bioavailability of physically dissolved  $O_2$  is excellent in profound anemia [12, 17, 18]), (3) infusion of norepinephrine (stabilization of coronary perfusion pressure during hemodilution [13]), and (4) artificial  $O_2$  carriers (maintenance of  $CaO_2$  despite reduced hematocrit [19, 20]). In contrast, anemia tolerance is decreased by (1) hypovolemia (reduction of microcirculatory  $O_2$  supply), (2) profound anesthesia (pharmacologic reduction of the cardiac output response to hemodilution [21, 22]) and (3) by coronary artery disease (reduced coronary flow reserve [23]).

During general anesthesia, NMB is usually applied to facilitate orotracheal intubation and mechanical ventilation and many surgical procedures (e.g. abdominal or thoracic surgery) require NMB. Whether the pharmacologic reduction of skeletal muscular tone by NMB has an impact on anemia tolerance has been of minor interest and has not yet been investigated.

Considering that skeletal muscular mass comprises 30–40% of total body mass [24] and that the cross-bridge cycle of muscular contraction is highly energy-dependent [25], it should be expected that NMB reduces skeletal  $mVO_2$  and should therefore decrease total body  $O_2$  demand.

In part, this assumption is supported by our data. Actually, the results of our pilot experiments clearly demon-



**Fig. 4.**  $Hb_{crit}$  investigated as the primary endpoint of the present study. As the hemodilution protocol could be performed more extensively in the Roc group, a critical limitation of total body  $O_2$  supply was met at a significantly lower  $Hb_{crit}$  ( $2.4 \pm 0.5$  g/dl vs.  $3.2 \pm 0.7$  g/dl,  $p < 0.05$  vs. Sal).

strate that NMB decreased  $mVO_2$ , regional blood flow to the skeletal muscle and muscular  $O_2$  extraction beyond their baseline values. Consistently, the reversal of NMB with sugammadex – a  $\gamma$ -cyclodextrin-based chelator with a specific affinity to steroidal relaxants (rocuronium > vecuronium > pancuronium) [26] – elevated these parameters and their values returned to the baseline level (fig. 2; table 1).

NMB had only a moderate effect on the total body level. After administration of rocuronium bromide, total body  $O_2$  extraction decreased and the negative  $VO_2$  difference to baseline ( $\Delta VO_2$ ) in the Roc group (vs. a positive value of  $\Delta VO_2$  in the Sal group) indicates a slight effect on total body  $VO_2$  (fig. 3). Apart from that, rocuronium bromide elicited notable hemodynamic effects, as very high doses were needed to achieve complete NMB (table 2).

**Table 3.** Limits of acute normovolemic anemia as reflected by the individual  $Hb_{crit}$  in different species

Author	Species	Anesthesia	FiO <sub>2</sub>	Plasma substitute	Identification of $Hb_{crit}$	$Hb_{crit}$ g · dl <sup>-1</sup>
Fontana et al. [37]	man (child)	isoflurane, sufentanil, vecuronium	1.0	albumin	decay of VO <sub>2</sub> (retrospectively)	2.1
Van Woerkens et al. [38]	man (84 years)	enflurane, fentanyl, pancuronium	0.4	gelatin	decay of VO <sub>2</sub> (retrospectively)	4
Zollinger et al. [39]	man (58 years)	propofol, fentanyl, pancuronium	1.0	gelatin	ST segment depression	~1.1
Cain [4]	dog	pentobarbital	0.21	dextran	decay of VO <sub>2</sub> (retrospectively)	3.3
Perez-de-Sá et al. [16]	pig	isoflurane, fentanyl, midazolam, vecuronium	0.5	dextran	decay of VO <sub>2</sub> (retrospectively)	2.3 ± 0.2
Meier et al. [12]	pig	propofol, fentanyl	0.21	HES	decay of VO <sub>2</sub> (prospectively)	3.1 ± 0.4
Pape et al. [17]	pig	propofol, fentanyl, midazolam, pancuronium	0.21	HES	decay of VO <sub>2</sub> (prospectively)	2.4 ± 0.4
Kemming et al. [18]	pig	midazolam, morphine, pancuronium	0.21	HES	ST segment depression	2.6 ± 0.3
Meisner et al. [19]	pig	diazepam, morphine, pancuronium	0.21	albumin	ST segment depression	2.0 ± 0.8
Meier et al. [13]	pig	propofol, fentanyl, pancuronium	0.21	HES	decay of VO <sub>2</sub> (prospectively)	2.6 ± 0.4
Pape et al. [15]	dog	propofol, midazolam, fentanyl, pancuronium	0.21	HES	decay of VO <sub>2</sub> (prospectively)	2.7 ± 0.5
Van der Linden [40]	dog	thiopental, ketamine, pancuronium	0.21	HES versus gelatin	decay of VO <sub>2</sub> (retrospectively)	3.6 ± 0.8 3.5 ± 1.5
Van der Linden [21]	dog	thiopental, ketamine (high dose), pancuronium thiopental, ketamine (low dose), pancuronium	0.21	HES	decay of VO <sub>2</sub> (retrospectively)	3.7 ± 1.4 2.5 ± 0.6

While the transient increase in pulmonary vascular resistance, mean pulmonary artery pressure and pulmonary capillary wedge pressure might have been evoked by histamine release, the increases in heart rate and CI are probably caused by vagolytic effects, which have been described for steroidal relaxants like pancuronium or rocuronium [27].

According to Fick's principle, the decrease in total body O<sub>2</sub> extraction immediately after administration of rocuronium might be interpreted as a response to increased CI – always presumed that VO<sub>2</sub> remains constant. However, Muldoon and Theye [28] reported already in 1969 that whole-body VO<sub>2</sub> was decreased in dogs paralyzed with d-tubocurarine, and some clinical studies investigating the effects of NMB on VO<sub>2</sub> reported a reduction of total-body VO<sub>2</sub> and energy expenditure by NMB in sedated and mechanically ventilated children [8] and in adults suffering acute respiratory failure [7], severe cerebral trauma [29] or burn injuries [30]. Inconsistently, other investigators found no effects of NMB on VO<sub>2</sub> in deeply sedated intensive care unit patients (Ramsay score 5) [31] or in anesthetized patients undergoing cardiac surgery [32].

The potential of NMB to reduce total body VO<sub>2</sub> might therefore depend on the depth of anesthesia. The anesthesia regimen applied in the present study (propofol, midazolam and fentanyl) has already been used in several of our previous investigations [13, 14, 17]. To the author's experience, this anesthesia regimen has proven appropriate for pigs, as it provided sufficient anesthetic depth without severely compromising cardiovascular function.

Under the anesthetic regimen applied in the present study, skeletal muscular activity did not substantially contribute to total body VO<sub>2</sub>, although NMB did reduce mVO<sub>2</sub>, muscular O<sub>2</sub> extraction and regional blood flow to the skeletal muscle.

Physiologically, blood flow to and within the skeletal muscle is regulated by the endothelial/vascular smooth muscle unit, which permits the physiological adaptation of regional O<sub>2</sub> delivery to the actual metabolic demand [33]. At the site of microcirculation, convective and diffusive O<sub>2</sub> transport is therefore enhanced in the contracting muscle, when compared with the skeletal muscle at rest [34]. Moreover, blood flow to the working muscle seems to be augmented during acute anemia: in humans

undergoing moderate hemodilution and submaximal muscular exercise, the increase in muscular blood flow was more strongly pronounced than in controls with physiologic arterial O<sub>2</sub> content [35]. This finding suggests that particularly in acute anemia, the elimination of muscular activity reduces skeletal muscular blood flow, thereby enabling a redistribution of O<sub>2</sub> delivery in favor of vital organs.

In summary, our data indicate that complete NMB increased the tolerance of acute normovolemic anemia in anesthetized pigs. The underlying mechanism most likely involves a reduction of skeletal muscular metabolic O<sub>2</sub> demand. Although paralyzed individuals may tolerate significantly lower Hb concentrations than nonparalyzed, a blood transfusion in clinical practice would usually be initiated before anemia tolerance is exhausted, especially since the individual anemia tolerance is strongly influenced by comorbidity including cardiovascular diseases or critical illness. However, an allogeneic blood transfusion may occasionally be impossible (e.g. refusal of blood transfusion for religious reasons, bridging an unexpected massive blood loss until compatible blood products become available for transfusion, prehospital trauma care) and very severe levels of acute normovolemic anemia have to be tolerated in these particular situations. In such extreme conditions, the augmentation of anemia tolerance by NMB might play an adjuvant role in maintaining DO<sub>2</sub> and oxygenation of vital organs despite extremely low Hb levels.

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## Appendix

### Calculations

Body surface area (BSA) was calculated according to Holt et al. [36] from body weight (BW) and a species-dependent constant (k = 9 for pigs):

$$BSA = k \cdot BW^{\frac{2}{3}} \left( m^2 \right) \quad (1)$$

CI was calculated as:

$$CI = \frac{CO}{BSA} \left( \frac{l}{min \cdot m^2} \right) \quad (2)$$

where CO = cardiac output.

Stroke volume index (SVI) was calculated as:

$$SVI = \frac{CI}{HR} \left( \frac{l}{m^2} \right) \quad (3)$$

where HR = heart rate.

Systemic and pulmonary vascular resistance indices were calculated as:

$$SVRI = \frac{(MAP - CVP) \cdot 79.9}{CI} \left( \frac{dyn \cdot s}{cm^5 \cdot m^2} \right) \quad (4)$$

and

$$PVRI = \frac{(MPAP - PCWP) \cdot 79.9}{CI} \left( \frac{dyn \cdot s}{cm^5 \cdot m^2} \right) \quad (5)$$

respectively, where SVRI = systemic vascular resistance index, PVRI = pulmonary vascular resistance index, MAP = mean aortic pressure, CVP = central venous pressure, MPAP = mean pulmonary artery pressure, and PCWP = pulmonary capillary wedge pressure.

Coronary perfusion pressure (CPP) was calculated as:

$$CPP = DAP - PCWP \text{ (mm Hg)}, \quad (6)$$

where CPP = coronary perfusion pressure, DAP = diastolic aortic pressure, and PCWP = pulmonary capillary wedge pressure.

Arterial and mixed venous O<sub>2</sub> contents (CaO<sub>2</sub> and CvO<sub>2</sub>) were calculated as:

$$CaO_2 = 1.34 \cdot [Hb] \cdot SaO_2 + 0.0031 \cdot paO_2 \left( \frac{ml}{dl} \right) \quad (7)$$

and

$$CvO_2 = 1.34 \cdot [Hb] \cdot SvO_2 + 0.0031 \cdot pvO_2 \left( \frac{ml}{dl} \right) \quad (8)$$

where SaO<sub>2</sub> and SvO<sub>2</sub> = arterial and mixed venous O<sub>2</sub> saturation and paO<sub>2</sub> and pvO<sub>2</sub> = arterial and mixed venous O<sub>2</sub> partial pressure.

O<sub>2</sub> delivery was indexed to BSA:

$$DO_2I = CI \cdot CaO_2 \left( \frac{ml}{min \cdot m^2} \right) \quad (9)$$

O<sub>2</sub> extraction ratio was calculated as:

$$O_2 - ER = \frac{CaO_2 - CvO_2}{CaO_2} \cdot 100(\%) \quad (10)$$

The relative difference of VO<sub>2</sub> to baseline after treatment was calculated as

$$\Delta VO_2 = \left( \frac{VO_{2Treatment}}{VO_{2Baseline}} \right) \cdot 100 - 100(\%) \quad (11)$$

Skeletal mVO<sub>2</sub> was calculated as:

$$mVO_2 = Q \cdot \frac{(CaO_2 - CfvO_2)}{100} \left( \frac{ml}{min} \right) \quad (12)$$

where Q = femoral arterial blood flow, CaO<sub>2</sub> and CfvO<sub>2</sub> = arterial and femoral venous O<sub>2</sub> content.



For induction and maintenance of anesthesia, the following drugs were administered:

Ketamine (Ketavet™, Parke-Davis, Berlin, Germany)  
Midazolam (Midazolam™, Ratiopharm, Ulm, Germany)  
Propofol (Propofol™, Braun, Melsungen, Germany)  
Fentanyl (Fentanyl™, Janssen, Neuss, Germany)  
Electrolyte solution (Tutofusin™, Baxter, Unterschleissheim, Germany)

Rocuronium bromide (Esmeron™, Essex Pharma, Oberschleissheim, Germany)

Sugammadex (Bridion™, Essex Pharma, Oberschleissheim, Germany)

The hemodilution protocol was performed with HES 130/0.4, Voluven™, Fresenius Kabi, Bad Homburg, Germany.

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