Mechanical ventilation restores blood gas homeostasis and diaphragm muscle strength in ketamine/medetomidine-anaesthetized rats

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Abstract
In pre-clinical small animal studies with surgical interventions under general anaesthesia, animals are often left to breathe spontaneously. However, anaesthesia may impair respiratory functions and result in disturbed blood gas homeostasis. In turn, the disturbed blood gas homeostasis can affect physiological functions and thus unintentionally impact the experimental results. We hypothesized that short-term mechanical ventilation restores blood gas balance and physiological functions despite anaesthesia and surgical interventions. Therefore, we investigated variables of blood gas analyses and diaphragm muscle strength in rats anaesthetized with ketamine/medetomidine after tracheotomy and catheterization of the carotid artery under spontaneous breathing and after 20 min of mechanical ventilation following the same surgical intervention. Spontaneous breathing during general anaesthesia and surgical intervention resulted in unphysiological blood oxygen partial pressure (<65 mmHg) and carbon dioxide partial pressure (>55 mmHg). After subsequent short-term mechanical ventilation, blood gas partial pressures were restored to their physiological ranges. Additionally, diaphragm muscle strength of animals breathing spontaneously was lower compared to animals that received subsequent mechanical ventilation (P = 0.0063). We conclude that spontaneous breathing of rats under ketamine/medetomidine anaesthesia is not sufficient to maintain a physiological blood gas balance. Disturbed blood gas balance is related to reduced diaphragm muscle strength. Mechanical ventilation for only 20 min restores blood gas homeostasis and muscle strength. Therefore, monitoring and maintenance of blood gas balance should be conducted to ensure quality and relevance of small animal experiments.

KEYWORDS
blood gas homeostasis, diaphragm muscle strength, mechanical ventilation, spontaneous breathing

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INTRODUCTION

Small animal models are frequently used to investigate physiological and pathophysiological processes in the living organism. Such experiments often include complex surgical interventions (often realizable only during spontaneous breathing). For that purpose, general anaesthesia is applied to avoid surgical stress and pain (Uhlig, Krause, Koch, Gama de Abreu, & Spieth, 2015). However, general anaesthesia is associated with the risk of impaired gas exchange (Hedenstierna & Rothen, 2012). If physiological values are not within their physiological ranges, body functions may be substantially affected. In this regard, hypoxia due to insufficient respiration may adversely affect study results (Vender, Hand, Sedor, Tabor, & Black, 1995).

We hypothesized that the application of mechanical ventilation restores blood gas balance and normal physiological function after anaesthesia with spontaneous breathing for surgical intervention. Therefore, we investigated variables of blood gas analyses and diaphragm muscle strength in spontaneously breathing rats (SB) undergoing a surgical intervention under general anaesthesia or in animals that received subsequent short-term mechanical ventilation (MV).

METHODS

2.1 Ethical approval

All animal experiments were approved by the review board of the local ethic committee (Regierungspräsidium Freiburg im Breisgau, Germany; Ref. No.: 35-9185.81/G14/81) and carried out in accordance with the German law for animal protection, the animal care guidelines of the European Union and the journal’s principles and regulations (Grundy, 2015).

2.2 Animal experiments

Female Sprague–Dawley rats (330 ± 35 g, Janvier Labs, Saint Berthevin Cedex, France) were kept 5–7 days in a holding facility prior to the experiments for adaptation. Animals housed following 12/12 h day/night cycle and animals had free access to water and food until the experiment. All experiments started at the same time to avoid variations due to circadian rhythms. Anaesthesia was induced with an intramuscular injection of ketamine (100 mg kg−1; Medistar Arzneimittelvertrieb GmbH, Ascheberg, Germany) and medetomidine chloride (1 mg kg−1; Orion Pharma GmbH, Hamburg, Germany). Animals were placed in the supine position on a heating pad to maintain body temperature (∼36°C) and subjected to tracheotomy followed by catheterization of the carotid artery.

Following a randomization scheme, the animals (n = 5 per group) were either left to breathe spontaneously (SB group) ambient air during the surgical intervention or received subsequent mechanical ventilation for 20 min with a tidal volume of 8 ml (kg body weight)−1, positive end-expiratory pressure of 2 cmH2O and a inspiratory oxygen fraction (FiO2) of 0.3 after the surgical intervention under spontaneous breathing (MV group; Figure 1). Blood samples were taken after the surgical intervention (BGA 1 in both experimental groups) and after the mechanical ventilation period (BGA 2 for MV group). Blood gases, acid-base balance and cation concentrations were analysed using an automated blood gas analyser (Cobas b221, Roche, Indianapolis, IN, USA). Heart rate was measured continuously with a small animal physiological monitoring system (75-1501, Harvard Apparatus, Holliston, MA, USA) placed on the right hind paw.

At the end of each experiment, the animals were exsanguinated under deep anaesthesia and the diaphragm was excised. Maximal diaphragm muscle strength was determined as described elsewhere (Armbruster et al., 2011). In brief, the diaphragm was placed on a pressure measurement unit. The pressure measurement unit was filled with 1 ml of air to preload the diaphragm (Ppreload). Maximal diaphragm contraction was induced by electrostimulation and the generated pressure (Pdiaphragm) was measured. The diaphragm muscle strength was defined as Pdiaphragm/Ppreload.

2.3 Statistical analysis

The data of the arterial partial pressure of oxygen (Pao2), the arterial partial pressure of carbon dioxide (Paco2) and the diaphragm muscle strength are presented as box plots (median, upper and lower quartile, and whiskers for minimal and maximal values). The data of the physiological variables are presented as means ± SD. All data were tested for normal distribution and homogeneity of variances, followed by an unpaired Student’s t tests or Welch’s test in the case that the
FIGURE 1  Experimental timeline. BGA, blood gas analyses; MV, mechanical ventilation; SB, spontaneous breathing

TABLE 1  Variables of blood gas analysis

|                  | SB BGA 1 | MV BGA 1 | MV BGA 2 | P               |
|------------------|----------|----------|----------|-----------------|
| $P_{aO2}$        | 57 ± 6   | 58 ± 4   | 135 ± 4  | <0.0001*       |
| $P_{aCO2}$       | 60 ± 3   | 58 ± 5   | 37 ± 1   | 0.0016#        |
| pH               | 7.353 ± 0.028 | 7.332 ± 0.019 | 7.446 ± 0.055 | 0.0003*       |
| $[HCO_3^-]$ (mmol l$^{-1}$) | 27.2 ± 0.5 | 26.7 ± 0.5 | 26.5 ± 1.1 | 0.3408*        |
| Base excess (mmol l$^{-1}$) | 6.2 ± 0.6  | 5.5 ± 0.8  | 3.1 ± 0.9  | 0.0014*        |
| $[Na^+]$ (mmol l$^{-1}$) | 138.5 ± 2.2 | 138.6 ± 1.6 | 136.1 ± 1.8 | 0.0279*        |
| $[Ca^{2+}]$ (mmol l$^{-1}$) | 1.4 ± 0.04 | 1.4 ± 0.03 | 1.4 ± 0.1 | 0.0959*        |
| $[K^+]$ (mmol l$^{-1}$) | 5.5 ± 0.5  | 5.3 ± 0.3  | 6.0 ± 0.6  | 0.8692*        |

The blood gas partial pressures, the acid-base–balance and the electrolytes of the animals after spontaneous breathing during anaesthesia and surgical interventions (SB BGA 1 and MV BGA 1) or after subsequent 20 min of mechanical ventilation (MV BGA 2). No differences between SB BGA 1 and MV BGA 1 were found. Student’s $t$ test was used for comparison of the sum of SB BGA 1 and MV BGA 1 vs. MV BGA 2. *Student’s $t$ test; #Welch’s test.

3  | RESULTS

The surgical intervention took approximately 30 min in all animals. Heart rate was comparable between the SB and MV group (278 ± 17 and 265 ± 27 bpm, respectively, $P = 0.0698$). The animals of the SB group showed lower $P_{aO2}$ and higher $P_{aCO2}$ compared to the animals of the MV group.

Additionally, blood pH was lower, and base excess, $[Na^+]$ and bicarbonate $[HCO_3^-]$ were higher in the SB group compared to the MV group (Table 1). No differences were found for $[Ca^{2+}]$ and $[K^+]$ in blood between the groups.

The rats that received subsequent mechanical ventilation showed on average two-fold higher diaphragm muscle strength compared to the rats after spontaneous breathing (Figure 2). Non-linear regression showed a correlation between $P_{aO2}$ and the diaphragm muscle strength ($R^2 = 0.96$).

4  | DISCUSSION

The present study revealed that blood gas homeostasis plays an important role in the orchestration of muscle strength physiology. Muscle contraction and generation of muscle strength require complex physiological conditions and processes. For example, physiological concentrations of $Na^+$, $Ca^{2+}$ and $K^+$ are essential for muscle function (van Dronkelaar et al., 2018). Concentrations both too high and too low may reduce muscle strength (Grassi et al., 2002; Rowley & Kliman, 1960) by influencing a wide range of physiological processes such as...
as nerve signal transduction, molecular interactions or energy intake (Murtaugh et al., 2018). In our model, Ca\(^{2+}\) and K\(^{+}\) concentrations in blood were comparable. Na\(^{+}\) concentrations differed significantly between the experimental groups but remained in the physiological range. Therefore, we assume that the observed differences in Na\(^{+}\) concentration had probably no relevant effect in our study.

After spontaneous breathing, the animals showed pH at the lower physiological limit. Blood pH is closely linked to muscle tissue pH, and particularly low pH is known to reduce muscle strength (Debold, Beck, & Warshaw, 2008). Among others, blood pH is affected by \(P_{\text{aCO2}}\). We assume that the differences in blood pH most likely originated from respiratory efficiency/inefficiency. Accordingly, rats that breathed spontaneously during anaesthesia with surgical intervention showed unphysiologically high \(P_{\text{aCO2}}\) values (all above 56 mmHg). Additionally, all spontaneously breathing rats showed unphysiologically low \(P_{\text{aO2}}\) values (all below 65 mmHg). In contrast, for animals that received mechanical ventilation, the \(P_{\text{aCO2}}\) and \(P_{\text{aO2}}\) levels in blood were in their physiological ranges. Interestingly, animals with restored blood gases homeostasis (MV group) showed higher diaphragm muscle strength compared to animals with unphysiological blood gases (SB group). This leads to the assumption that diaphragm muscle strength is related to the homeostasis of the blood gases. This speculation is supported by the high correlation between \(P_{\text{aO2}}\) and diaphragm muscle strength. The exponential relation suggests that oxygen saturation above a certain threshold is needed for optimal muscle function.

It is worth noting that differences of \(P_{\text{aO2}}\) between the experimental groups could not only be explained by the higher \(F_{\text{I O2}}\) (0.3) during mechanical ventilation. Actually a \(F_{\text{I O2}}\) value of 0.21 should result in a \(P_{\text{aO2}}\) of ca 100 mmHg. Therefore, low \(P_{\text{aO2}}\) could be an indicator for atelectasis and related pulmonary shunt in spontaneous breathing animals under general anaesthesia (Petersson & Glenny, 2014). Therefore, we assume that the two-fold higher \(P_{\text{aO2}}\) could rather be attributed to respiratory efficiency due to mechanical ventilation than to the higher \(F_{\text{I O2}}\). In addition, further factors, for example micro-perfusion rates of diaphragm, may influence muscle contractility (Brancatisano, Amis, Tuly, Kelly, & Engel, 1991). We assume that even comparable perfusion rates between SB and MV would not be sufficient to maintain diaphragm muscle strength in the SB group due to the existing unphysiological blood gas levels. It should be noted that mechanical ventilation could in turn cause diaphragm weakness (Dres & Demoule, 2020; Levine et al., 2008). Therefore, the duration of mechanical ventilation should be kept as short as required to restore blood gas balance.

The present study revealed that spontaneous breathing during surgery in ketamine/medetomidine-anaesthetized rats is not sufficient to maintain an adequate blood gas balance. The outcomes of physiological measurements (in particular the diaphragm muscle strength) appear to be strongly related to blood gas homeostasis. Therefore, monitoring and proper respiratory support are a potential tools to increase relevance and quality of physiological measurements in animal experiments.

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**COMPETING INTERESTS**

The authors declare no conflict of interest.

**AUTHOR CONTRIBUTIONS**

Study design: C.W. and S.G.S. Performing of animal experiments: C.W. and S.G.S. Data analysis/interpretation: C.W., G.U., J.S., S.S. and S.G.S. Writing of manuscript: C.W., G.U., J.S., S.S. and S.G.S. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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