

# Effects of human relaxin-2 (serelaxin) on hypoxic pulmonary vasoconstriction during acute hypoxia in a sheep model

René Schiffner<sup>1,2</sup>

Marius Nistor<sup>2</sup>

Sabine Juliane Bischoff<sup>3</sup>

Georg Matziolis<sup>1</sup>

Martin Schmidt<sup>4</sup>

Thomas Lehmann<sup>5</sup>

<sup>1</sup>Orthopaedic Department,

<sup>2</sup>Department of Neurology, <sup>3</sup>Central Animal Facility, <sup>4</sup>Institute for Biochemistry II, <sup>5</sup>Institute of Medical Statistics, Computer Sciences and Documentation Science, Jena University Hospital, Friedrich Schiller University, Jena, Germany

**Purpose:** Hypoxia induces pulmonary vasoconstriction with a subsequent increase of pulmonary artery pressure (PAP), which can result in pulmonary hypertension. Serelaxin has shown an increase of pulmonary hemodynamic parameters after serelaxin injection. We therefore investigated the response of pulmonary hemodynamic parameters after serelaxin administration in a clinically relevant model.

**Methods:** Six controls and six sheep that received 30 µg/kg serelaxin underwent right heart catheterization during a 12-minute hypoxia period (inhalation of 5% oxygen and 95% nitrogen) and subsequent reoxygenation. Systolic, diastolic, and mean values of both PAP (respectively, PAPS, PAPd, and PAPm) and pulmonary capillary wedge pressure (respectively, PCWPs, PCWPD, and PCWPm), blood gases, heart rate (HR), and both peripheral and pulmonary arterial oxygen saturation were obtained. Cardiac output (CO), stroke volume (SV), pulmonary vascular resistance (PVR), pulmonary arterial compliance (PAcompl), and systemic vascular resistance (SVR) were calculated.

**Results:** The key findings of the current study are that serelaxin prevents the rise of PAPS ( $p \leq 0.001$ ), PAPm, PCWPD, PCWPs ( $p \leq 0.03$ ), and PAPd ( $p \leq 0.05$ ) during hypoxia, while it simultaneously increases CO and SV ( $p \leq 0.001$ ). Similar courses of decreases of PAPm, PAPd, PAPS, CO, SVR ( $p \leq 0.001$ ), and PCWPD ( $p \leq 0.03$ ) as compared to hypoxic values were observed during reoxygenation. In direct comparison, the experimental groups differed during hypoxia in regard to HR, PAPm, PVR, and SVR ( $p \leq 0.03$ ), and during reoxygenation in regard to HR ( $p \leq 0.001$ ), PAPm, PAPS, PAPd, PVR, SVR ( $p \leq 0.03$ ), and PCWPD ( $p \leq 0.05$ ).

**Conclusion:** The findings of this study suggest that serelaxin treatment improves pulmonary hemodynamic parameters during acute hypoxia.

**Keywords:** hypoxic pulmonary vasoconstriction, pulmonary hemodynamics, relaxin-2, hypoxia, serelaxin, right heart catheterization

## Introduction

During states of hypoxia, pulmonary circulation undergoes several changes and thereby deviates from systemic circulation. While lack of oxygen has direct vasodilating effects on systemic arterioles, it constitutes the most important vasoconstricting trigger acting on the pulmonary arterioles.<sup>1</sup> These physiologic response mechanisms account for the underlying pathogenic reasons for the development of pulmonary hypertension as well.<sup>2-5</sup> Intermittent hypoxia results in an increase of pulmonary artery pressure (PAP) and alters the general pulmonary hemodynamic physiology, for example by reducing pulmonary arterial compliance (PAcompl), increasing pulmonary vascular resistance (PVR) and systemic vascular resistance (SVR), and decreasing cardiac output (CO), as

Correspondence: René Schiffner  
Orthopaedic Department, Jena University  
Hospital, Friedrich Schiller University,  
Campus Eisenberg, Klosterlausnitzer  
Straße 81, D-07607 Eisenberg, Germany  
Tel +49 366 918 1292  
Fax +49 366 918 1029  
Email rene.schiffner@med.uni-jena.de

reported in various animal and human studies.<sup>6–14</sup> Pulmonary vasoconstriction, with its subsequent pulmonary hemodynamic alterations, is a contributing factor in the development of heart failure.<sup>15</sup>

The therapeutic administration of a recombinant form of human relaxin-2 (serelaxin; Novartis Pharma, Basel, Switzerland) is still controversially discussed.<sup>16–18</sup> In the RELAX-AHF study, cardiovascular and all-cause mortality were reduced 37% in patients treated with serelaxin.<sup>16</sup> The follow-up study RELAX-AHF 2 failed to substantiate these results.<sup>18</sup> Complete data of the RELAX-AHF 2 study are not available currently. Relaxin-2 modulates the endothelial vasodilatory function in humans; the same effects are inducible in nonpregnant animals as well.<sup>19,20</sup>

Serelaxin furthermore increases arterial compliance and improves pulmonary hemodynamics.<sup>21–23</sup> Its positive influences on pulmonary hemodynamics have already been shown in patients with pulmonary hypertension.<sup>15</sup> Nonetheless, specific responses of hemodynamic parameters during states of hypoxia after serelaxin administration have not been investigated so far. An examination of the effects of serelaxin under this specific condition seems necessary though, since it is conceivable that serelaxin could constitute a negative influence in these situations. Since serelaxin triggers an endothelial modulation with a subsequent vasodilation, cardiopulmonary instability appears possible in cases of severe clinical situations, for example hypoxia.

## Materials and methods

### Animal care, surgical instrumentation, anesthesia, and experimental protocol

Approval for the experiment was obtained from the Thuringia Animal Welfare Committee, (Bad Langensalza; permission number TVV 02-056/13 valid from December 13, 2013 until December 20, 2016) and all procedures were conducted in conformity with the Animal Welfare Act, and the ARRIVE guidelines<sup>82</sup> were followed. Twelve 2–6 year old female, nonpregnant Merino long wool sheep, weighing  $84.7 \pm 6.2$  kg, underwent surgery in accordance with the Guide for the Care and Use of Laboratory Animals.<sup>24</sup> We have previously described the surgical instrumentation, anesthesia, data recording, and blood gas measurements in detail.<sup>25</sup> Briefly, after food withdrawal for 24 hours, anesthesia was induced by intramuscular injection of ketamine and was maintained via inhalation of 1.5% isoflurane over the course of the entire experiment. In a left lateral position, vascular catheters were inserted into the carotid artery (for blood sampling, blood pressure measurement), the left jugular

vein (for intraoperative administration of analgesics), and the right jugular vein with a Swan–Ganz catheter (for blood sampling and assessment of pulmonary hemodynamics in the pulmonary artery). The right heart data acquisition was continuously performed and has been described previously.<sup>26</sup> We used a heart catheter station (evoelement®; Schwarzer, Heilbronn, Germany) for data recording and to analyze the primary data in connection to time points (eg, heart rate [HR], PAP, pulmonary capillary wedge pressure [PCWP]).

Subsequent to 10 minutes of baseline recordings, during oxygenation ensued with 50% oxygen, six sheep (control group) received a bolus injection of 20 mL saline (Isotonische Kochsalzlösung®; Fresenius, Bad Homburg, Germany) and six sheep (serelaxin-treated group) received an injection of 30 µg/kg serelaxin (0.1–0.15 mL stock solution) diluted in saline (serelaxin).

### Experimental protocol of hypoxia

Hypoxia was induced by inhalation of 5% oxygen and 95% nitrogen and was maintained for 12 minutes; subsequently, reoxygenation ensued with 50% oxygen over 20 minutes under constant monitoring of breathing and heart frequency, blood pressure, blood gas concentrations, body temperature, oxygen saturation, and shock index values.

### Pulmonary hemodynamics

During the right heart catheterization, the systolic, diastolic, and mean PAP (PAPs, PAPd, and PAPm), systolic, diastolic, and mean pulmonary capillary wedge pressure (PCWPs, PCWPd, and PCWpm), blood gases, and HR were recorded as described previously.<sup>26</sup> CO was calculated with Fick's method,<sup>27</sup> PVR was calculated as  $(PAPm - PCWpm)/CO$ .<sup>28</sup> SVR was calculated as mean arterial blood pressure (MABP)/CO, the stroke volume (SV) was calculated as  $CO/HR$ , and PAcompl was calculated as  $(PAPs - PAPd)/SV$ .<sup>28</sup>

### Statistical analysis

Linear mixed regression models were fitted to compare the trend of pulmonary hemodynamic parameters between both groups. This approach allows accounting for the correlation of the repeated measurements of an animal. Independent variables of the models are group (serelaxin/control) and time of measurement, as well as their interaction. For each condition (hypoxia, no hypoxia), a separate model was calculated. Regression coefficients of the models were presented to describe the average change of pulmonary hemodynamic parameters per minute. The significance level was set to  $\alpha=0.05$ . All statistical analyses were performed with SPSS

22.0 (IBM Corporation, Armonk, NY, USA). Data are given as mean differences  $\pm$  standard error of means or describe a trend over the time of each represented variable per minute. As some data sets were normally distributed all quantitations are presented as box plots, where boxes represent the 25th and 75th percentiles, respectively. Medians are indicated by horizontal lines. Whiskers indicate 10th and 90th percentiles, respectively.

## Results

### Blood parameters and vital parameters under serelaxin treatment during hypoxia and reoxygenation

Arterial blood gases and lactate were within their physiological ranges before the onset of controlled hypoxia with subsequent reoxygenation (Table 1). Hypoxia induced a decrease of pH and  $pO_2$ , and an increase of lactate ( $p<0.05$ , Table 1). Reoxygenation normalized pH and  $pCO_2$ , and  $pO_2$  was restored to baseline. No differences were observed between the control and the serelaxin-treated group.

During hypoxia, no significant changes of the MABP were observed over time and between the groups. During reoxygenation, the serelaxin-treated group exhibited slightly decreased MABP values directly after the hypoxic episode ( $p=0.015$ ); nonetheless, no significant differences were observed between the groups (Figure 1A).

During the hypoxic intervention, the HR was significantly lower in the control group than in the serelaxin-treated group (mean difference =48.4 beats per minute [bpm],  $p=0.015$ ). On average, hypoxia significantly increased the HR by 2.9 bpm in the serelaxin-treated group ( $p<0.001$ ). During reoxygenation, the HR values of the control group were again significantly lower than in the serelaxin-treated group (mean difference

=65.9 bpm,  $p<0.001$ ). On average, reoxygenation significantly decreased the HR by 2.4 bpm in the serelaxin-treated group ( $p<0.001$ ), as compared to the HR values measured at the end of the hypoxic episode. No significant changes of the HR were observed over time during hypoxia and reoxygenation in the control group (Figure 1B).

The femoral arterial and pulmonary arterial oxygen saturation exhibited similar courses of decreases in both groups over the course of the hypoxia, and thereafter similar courses of increases under reoxygenation in both groups ( $p<0.001$ ) (Figure 2A, B).

### Effects of serelaxin on hemodynamic parameters and vascular resistance during hypoxia and reoxygenation

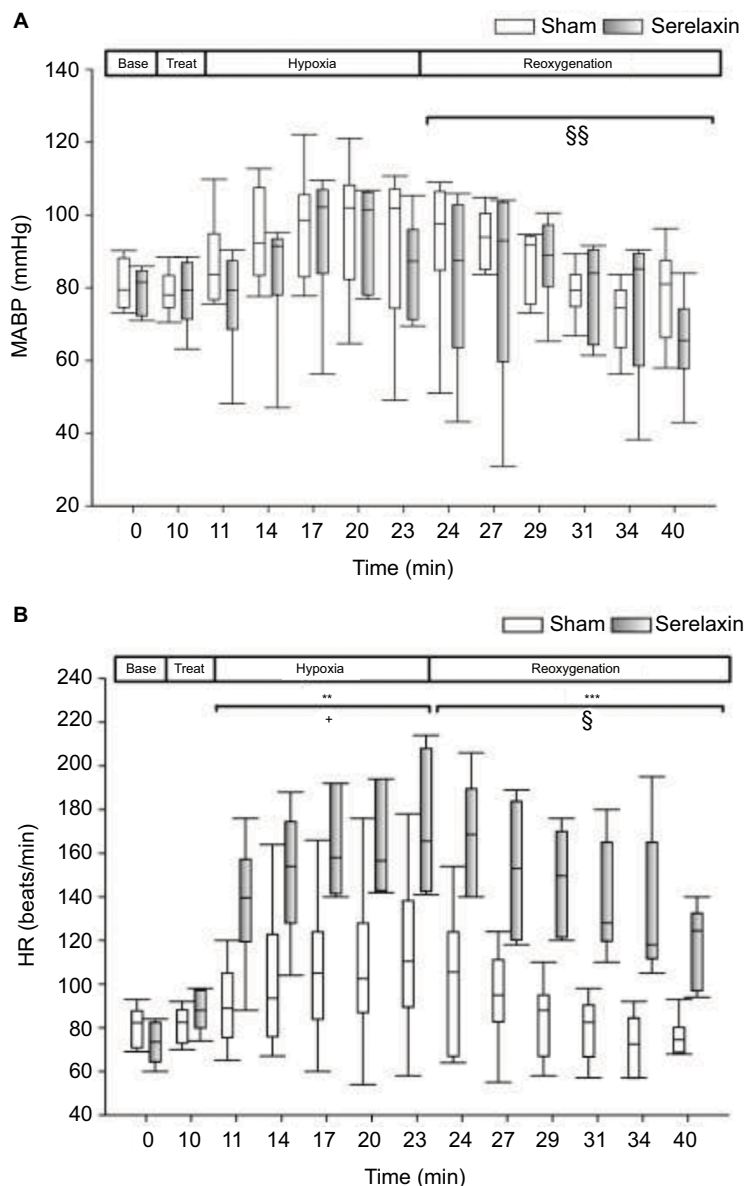
Analysis of the PAP revealed significant differences regarding the development of the PAPm, PAPs, and PAPd during the observation period in the serelaxin-treated group as compared to the control group. No significant baseline differences exist between the groups. During hypoxia, PAPm was significantly lower in the serelaxin-treated group than in the control group (mean difference =14.1 mmHg,  $p=0.019$ ). On average, PAPm significantly increased by 0.6 mmHg per minute during hypoxia in the control group ( $p=0.002$ ). During reoxygenation, PAPm remained significantly higher in the control group than in the serelaxin-treated group (mean difference =11.6 mmHg,  $p=0.003$ ). During reoxygenation, the PAPm significantly decreased by 0.8 mmHg per minute in the control group ( $p<0.001$ ) and 0.3 mmHg per minute in the serelaxin-treated group ( $p=0.001$ ) (Figure 3A). The PAPs of the control group significantly increased by 0.9 mmHg per minute during hypoxia ( $p=0.001$ ). During reoxygenation, PAPs remained significantly lower in the serelaxin-treated

**Table 1** Blood parameters during hypoxia

	Blood parameters							
	Control group				Serelaxin-treated group			
	Basal	1-min hypoxia	12-min hypoxia	20 min after reoxygenation	Basal	1-min hypoxia	12-min hypoxia	20 min after reoxygenation
pH	7.4 ( $\pm 0.08$ )	7.37 ( $\pm 0.13$ )	7.33* ( $\pm 0.04$ )	7.3 ( $\pm 0.16$ )	7.4 ( $\pm 0.06$ )	7.43 ( $\pm 0.05$ )	7.32* ( $\pm 0.09$ )	7.37 ( $\pm 0.04$ )
$pCO_2$ (kPa)	6 ( $\pm 0.9$ )	6.6 ( $\pm 0.4$ )	5.9 ( $\pm 0.8$ )	6.2 ( $\pm 0.9$ )	5.7 ( $\pm 1.1$ )	5.7 ( $\pm 0.9$ )	4.9 ( $\pm 0.3$ )	6.1 ( $\pm 0.6$ )
$pO_2$ (kPa)	10.2 ( $\pm 2.6$ )	12.3 ( $\pm 2.8$ )	2.8* ( $\pm 0.5$ )	12.1 ( $\pm 3$ )	10.8 ( $\pm 1.8$ )	13.4 ( $\pm 3.3$ )	2.7* ( $\pm 0.3$ )	10.6 ( $\pm 1.6$ )
Lactate (mmol/L)	2.2 ( $\pm 0.7$ )	1.7 ( $\pm 0.4$ )	4.7* ( $\pm 1.0$ )	3.5 ( $\pm 0.7$ )	2.9 ( $\pm 2$ )	1.9 ( $\pm 1$ )	6.1* ( $\pm 1.3$ )	3.1 ( $\pm 1.8$ )
Glucose (mmol/L)	3.5 ( $\pm 1.2$ )	3.4 ( $\pm 0.9$ )	6.9 ( $\pm 2.4$ )	8.4 ( $\pm 3.2$ )	3.2 ( $\pm 0.8$ )	3.2 ( $\pm 0.7$ )	4.6 ( $\pm 1$ )	4.9 ( $\pm 1.9$ )
BE (mmol/L)	3 ( $\pm 2.6$ )	3.7 ( $\pm 2.6$ )	2.9 ( $\pm 1.7$ )	0 ( $\pm 3$ )	2.4 ( $\pm 4.7$ )	3.7 ( $\pm 3.9$ )	3.7 ( $\pm 2.1$ )	2 ( $\pm 3$ )

**Notes:** Changes of blood parameters are listed. Blood samples were drawn from the arteria femoralis. Samples were taken at baseline (basal), immediately during 1-min hypoxia, during 12-min hypoxia, or 20 min after reoxygenation (total 40 min). All data are given as mean  $\pm$  SD, \* $p<0.05$ .

**Abbreviations:**  $pCO_2/pO_2$ , partial pressure of carbon dioxide/oxygen; BE, base excess.



**Figure 1** Effects of serelaxin during hypoxia and reoxygenation on mean arterial pressure and heart rate.

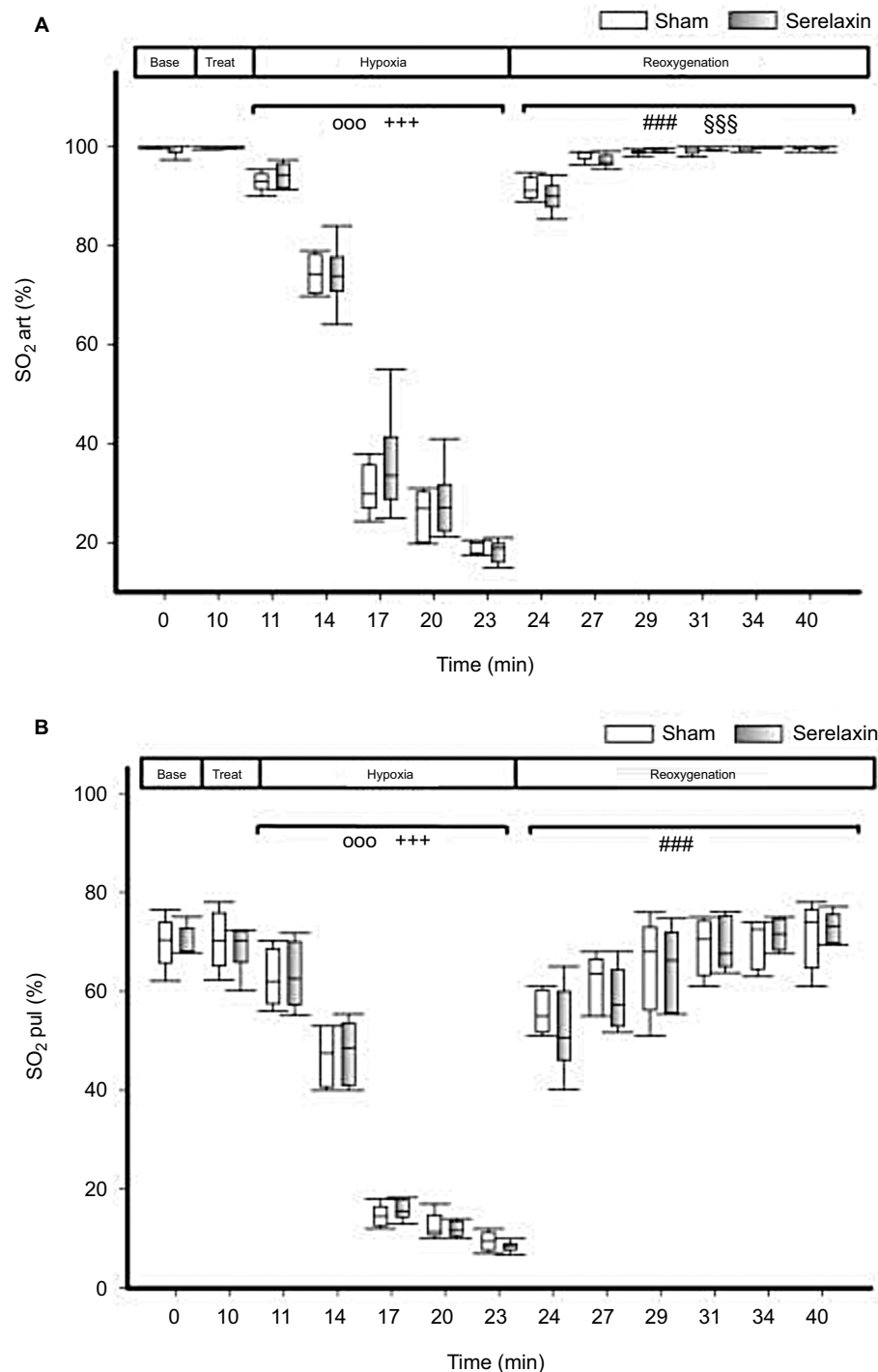
**Notes:** (A) MABP and (B) HR of the serelaxin-treated (gray symbols) or control group (sham, white symbols), respectively. \*\*\*,  $p \leq 0.001$ ; \*\*,  $p \leq 0.03$ , for comparison between the two experimental groups during hypoxia or reoxygenation, respectively. +,  $p \leq 0.05$ , for comparison of the serelaxin-treated group during hypoxia with baseline values. §§,  $p \leq 0.03$ ; §,  $p \leq 0.05$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group.

**Abbreviations:** MABP, mean arterial blood pressure; HR, heart rate.

group as compared to the control group (mean difference = 12.6 mmHg,  $p = 0.018$ ); PAPs decreased by 1.2 mmHg per minute in the control group ( $p < 0.001$ ) and by 0.7 mmHg per minute in the serelaxin-treated group ( $p = 0.014$ ) over the course of the reoxygenation period as compared to the values measured at the end of the hypoxic period ( $p < 0.001$ ) (Figure 3B). PAPd of the control group significantly increased by 0.5 mmHg per minute during hypoxia ( $p = 0.044$ ). PAPd of the serelaxin-treated group was significantly lower during the reoxygenation period as compared to the control group

(mean difference = 11.4 mmHg,  $p = 0.005$ ) and decreased by 0.1 mmHg per minute as compared to the values measured at the end of the hypoxic period ( $p = 0.001$ ). PAPd in the control group decreased by 0.6 mmHg per minute during reoxygenation compared to hypoxic values ( $p < 0.001$ ) (Figure 3C).

Analysis of the PCWP values revealed a somewhat similar situation, with significant differences of PCWPm, PCWPs, and PCWpd between both groups over the course of the observation period. No significant baseline differences exist between the groups. During hypoxia, the PCWPm of the



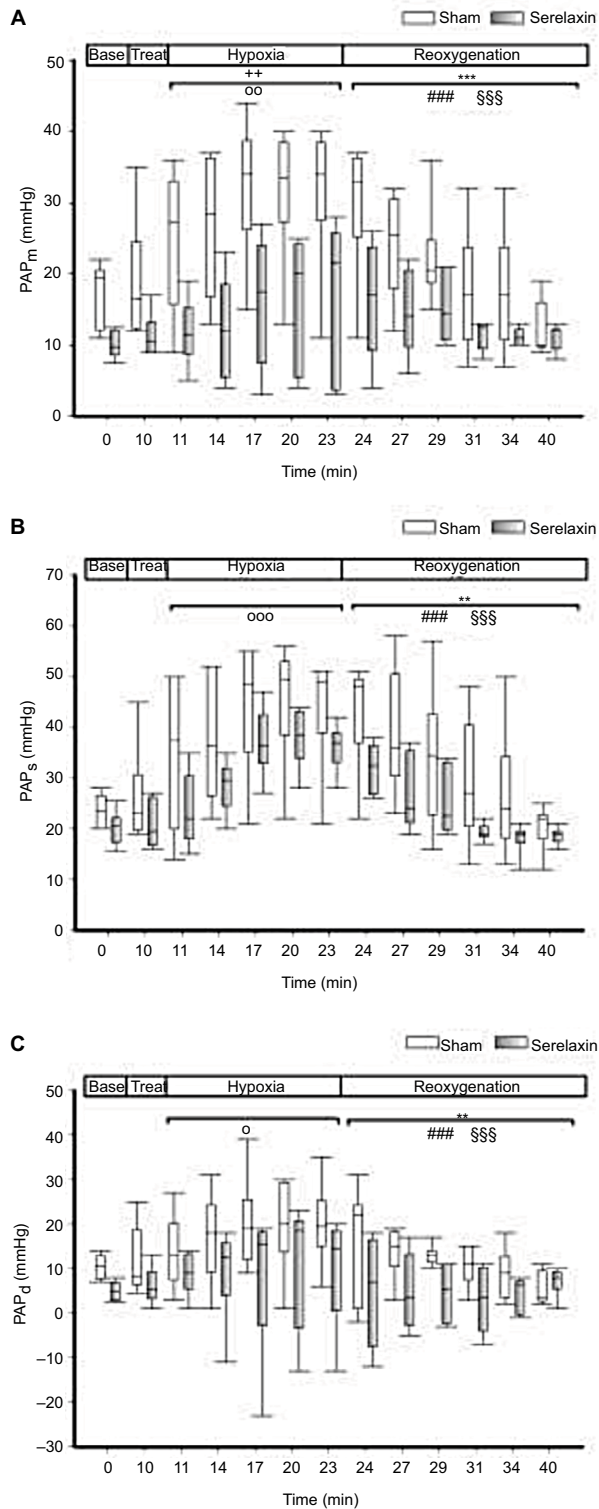
**Figure 2** Effects of serelaxin during hypoxia and reoxygenation on arterial blood gases.

**Notes:** (A) SO<sub>2</sub> art and (B) SO<sub>2</sub> pul. °°°,  $p \leq 0.001$ , for comparison of the control group during hypoxia with baseline values. +++,  $p \leq 0.001$ , for comparison of the serelaxin-treated group during hypoxia with baseline values. \$\$\$,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group. ###,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the control group.

**Abbreviations:** SO<sub>2</sub> art, femoral arterial oxygen saturation; SO<sub>2</sub> pul, pulmonary arterial oxygen saturation.

control group significantly increased by 0.4 mmHg per minute as compared to baseline ( $p=0.037$ ). During reoxygenation, PCWpm decreased by 0.6 mmHg per minute in the control group ( $p<0.001$ ) and by 0.2 mmHg in the serelaxin-treated group ( $p=0.037$ ) as compared to hypoxic values (Figure 4A).

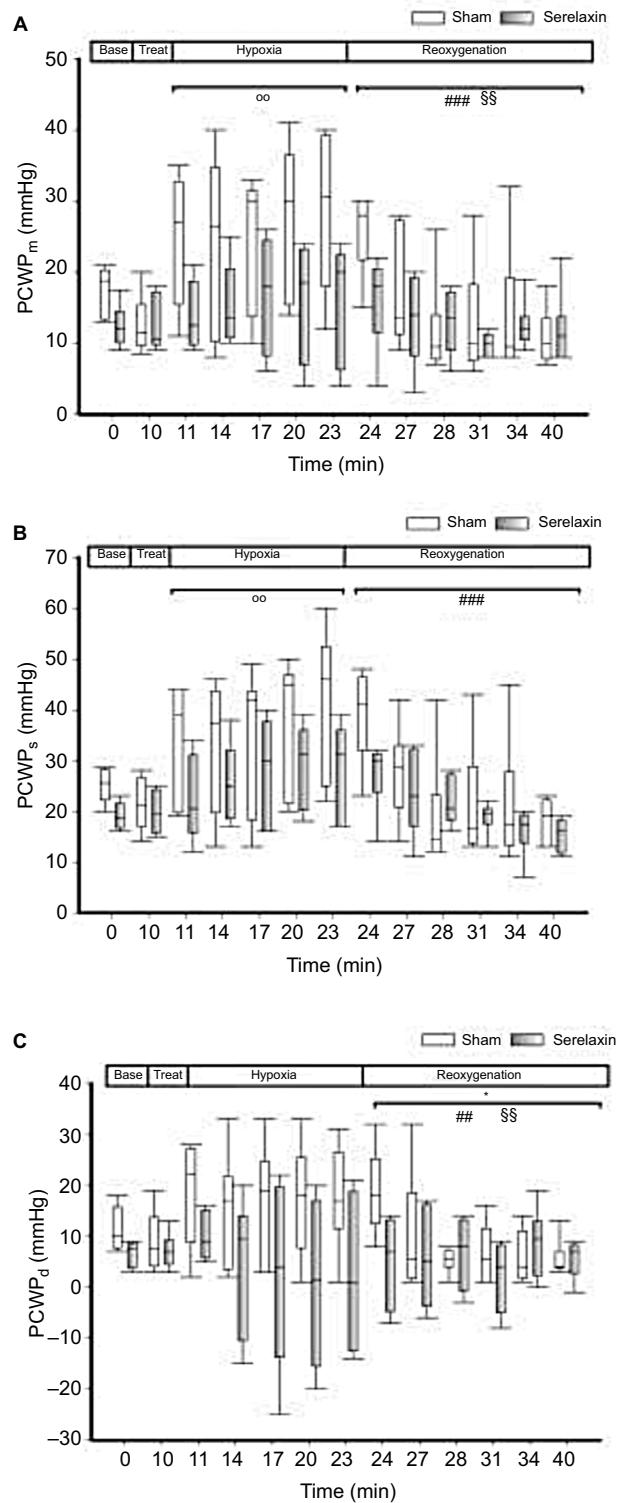
The PCWPs of the control group significantly increased by 0.7 mmHg per minute during hypoxia ( $p=0.005$ ), and decreased by 0.8 mmHg per minute ( $p<0.001$ ) during the reoxygenation; no significant changes were observed between the control group and the serelaxin-treated group (Figure 4B).



**Figure 3** Effects of serelaxin during hypoxia and reoxygenation on pulmonary artery pressure.

**Notes:** (A)  $PAP_m$ , (B)  $PAP_s$ , and (C)  $PAP_d$ , \*\*,  $p \leq 0.03$ , for comparison between the two experimental groups during hypoxia or reoxygenation, respectively. °°,  $p \leq 0.001$ ; °,  $p \leq 0.05$ ; °°,  $p \leq 0.03$ ; °°,  $p \leq 0.001$ , for comparison of the control group during hypoxia with baseline values. ###,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the control group. §§,  $p \leq 0.03$ ; §§§,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group.

**Abbreviations:**  $PAP_m$ , mean pulmonary artery pressure;  $PAP_s$ , systolic pulmonary artery pressure;  $PAP_d$ , diastolic pulmonary artery pressure.



**Figure 4** Effects of serelaxin during hypoxia and reoxygenation on pulmonary capillary wedge pressure.

**Notes:** (A)  $PCWP_m$ , (B)  $PCWP_s$ , and (C)  $PCWP_d$ , \*,  $p \leq 0.05$ , for comparison between the two experimental groups during hypoxia or reoxygenation, respectively. °°,  $p \leq 0.03$ , for comparison of the control group during hypoxia with baseline values. ###,  $p \leq 0.001$ ; ##,  $p \leq 0.03$ , for comparison of reoxygenation with hypoxia in the control group. §§,  $p \leq 0.03$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group.

**Abbreviations:**  $PCWP_m$ , mean pulmonary capillary wedge pressure;  $PCWP_s$ , systolic pulmonary capillary wedge pressure;  $PCWP_d$ , diastolic pulmonary capillary wedge pressure.

Regarding PCWpd, no significant changes could be observed between the groups during hypoxia; nonetheless, PCWpd of the serelaxin-treated group was significantly lower during the reoxygenation period as compared to the control group (mean difference =7.3 mmHg,  $p=0.049$ ), and increased by 0.1 mmHg per minute as compared to the hypoxic episode ( $p=0.018$ ). PCWpd of the control group decreased by 0.5 mmHg per minute during reoxygenation, as compared to the hypoxic episode ( $p=0.003$ ) (Figure 4C).

Analysis of the CO revealed no significant differences between the groups over the course of hypoxia and reoxygenation. CO significantly increased by 0.2 L per minute in the serelaxin-treated group during hypoxia. It decreased in both groups during reoxygenation as compared to hypoxic values; in the serelaxin-treated group by 0.2 L/min and in the control group by 0.1 L/min ( $p<0.001$ ) (Figure 5A). SV exhibited no significant differences between the groups over the course of hypoxia and reoxygenation. In the serelaxin-treated group, the SV significantly increased by 0.008 L/beat per minute during hypoxia ( $p<0.001$ ) and decreased by 0.003 L/beat per minute during reoxygenation ( $p<0.001$ ) (Figure 5B).

PVR of the serelaxin-treated group was significantly lower than that of the control group, both during hypoxia (mean difference =0.5 Wood's Unit [WU],  $p=0.024$ ) and reoxygenation (mean difference =0.8 WU,  $p=0.005$ ). On average, in the serelaxin-treated group the PVR significantly decreased by 0.05 WU per minute during reoxygenation as compared to hypoxic values ( $p=0.005$ ) (Figure 6A). SVR was significantly lower in the serelaxin-treated group as compared to the control group (mean difference =1.5 mmHg/[L/min],  $p=0.002$ ), especially during reoxygenation (mean difference =1.7 mmHg/[L/min],  $p=0.004$ ). On average, SVR significantly decreased by 0.1 mmHg/(L/min) in the serelaxin-treated group ( $p=0.023$ ), and by 0.2 mmHg/(L/min) in the control group ( $p<0.001$ ) during hypoxia compared to baseline values (Figure 6B). During reoxygenation, the SVR significantly increased by 0.1 mmHg/(L/min) in the serelaxin-treated group ( $p=0.001$ ), and by 0.2 mmHg/(L/min) in the control group ( $p<0.001$ ) as compared to hypoxic values (Figure 6B).

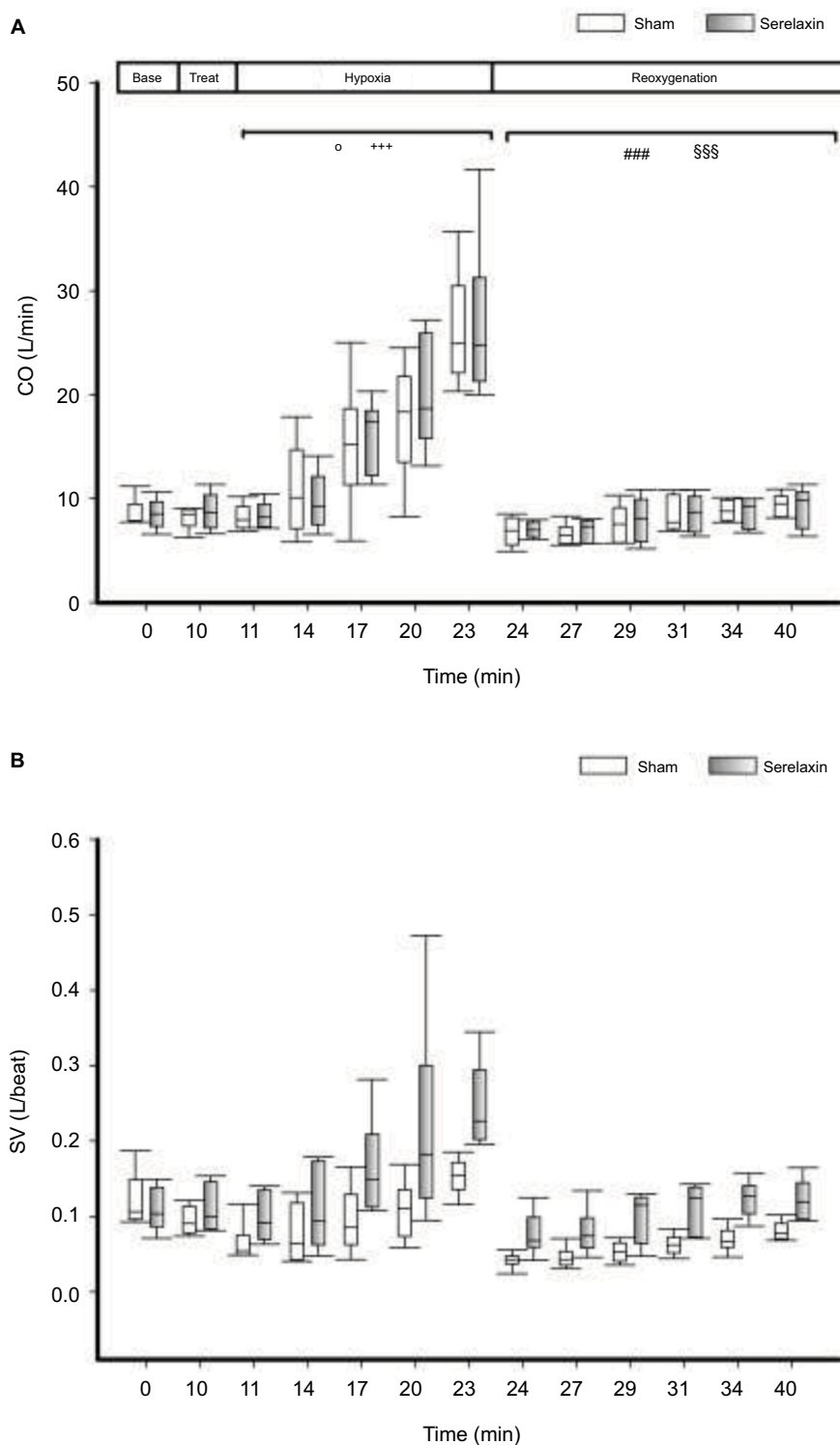
No differences between the PAcompl values of both groups could be observed during hypoxia. During reoxygenation, PAcompl of the serelaxin-treated group significantly decreased by 0.0003 L/mmHg per minute ( $p=0.021$ ) (Figure 6C).

## Discussion

The main new insights our experimental protocol revealed are that i) a decreased PAPm, ii) a decreased PVR, iii) and

a decreased SVR can be observed in a sheep model of acute hypoxic vasoconstriction after a bolus injection of serelaxin.

Various studies have shown an increase of CO subsequent to serelaxin application, which has been described in experiments featuring relaxin-2 treatment in rats,<sup>29</sup> sheep,<sup>26</sup> and in acute heart failure patients treatment with preserved left ventricular ejection fraction undergoing serelaxin treatment.<sup>15</sup> Without the influence of medication though, CO and SV are known to remain unaltered under hypoxic conditions.<sup>30</sup> Nevertheless, our results indicate that serelaxin quickly improves pulmonary hemodynamics, with no significant alterations of the global hemodynamics under hypoxic conditions. Furthermore, the results indicate that receptor-mediated changes occur regionally. Relaxin-2 is a very potent arterial vasodilator which exerts a rapid effect (within minutes) on the regional circulation in the brain and kidneys.<sup>31,32</sup> Animal studies have already described systemic vasodilatory effects of relaxin-2, with a subsequent increase of vascular compliance and improvements of peripheral vasodilatation and atrial inotropy.<sup>19,26,33,34</sup> The primary effect on inotropy has not been measured in this study. The positive chronotropic effects of relaxin-2 have been described previously.<sup>35</sup> The positive influence of serelaxin on pulmonary hemodynamics in patients with pulmonary hypertension has already been shown in a study utilizing right heart catheterization procedures.<sup>15</sup> The safe invasive procedure of right heart catheterization is the gold standard for hemodynamic assessments.<sup>36,37</sup> Although a previous study has already provided evidence that serelaxin improves pulmonary hemodynamics in humans with pulmonary hypertension during a right heart catheterization,<sup>15</sup> up to this point no study had been performed under acute hypoxic conditions. Our study provides evidence that similar positive effects can be observed under hypoxic conditions in a sheep model after serelaxin administration. Various studies have revealed that hypoxic conditions result in an increase of PAP, PCWP, and an decrease of PVR, SVR.<sup>38-45</sup> We utilized the large animal sheep model since its cardiovascular physiology is very similar to that of humans, which makes it a suitable model to investigate different conditions of the pulmonary vascular tree.<sup>46,47</sup> These kinds of investigations necessitate a large animal model, since the otherwise oftentimes utilized rodent model is unsuitable due to the ability of rodents to adapt to low oxygen concentrations.<sup>1</sup> The animals received a bolus injection of serelaxin equivalent to a daily human dose.<sup>48</sup> This represents a consciously chosen high dosage, which would be administered over a period of 24 hours in humans.<sup>49</sup> While this dose does therefore not represent actual serelaxin prescription schemes for heart failure,<sup>48</sup> it ensured



**Figure 5** Effects of serelaxin during hypoxia and reoxygenation on cardiac output and stroke volume.

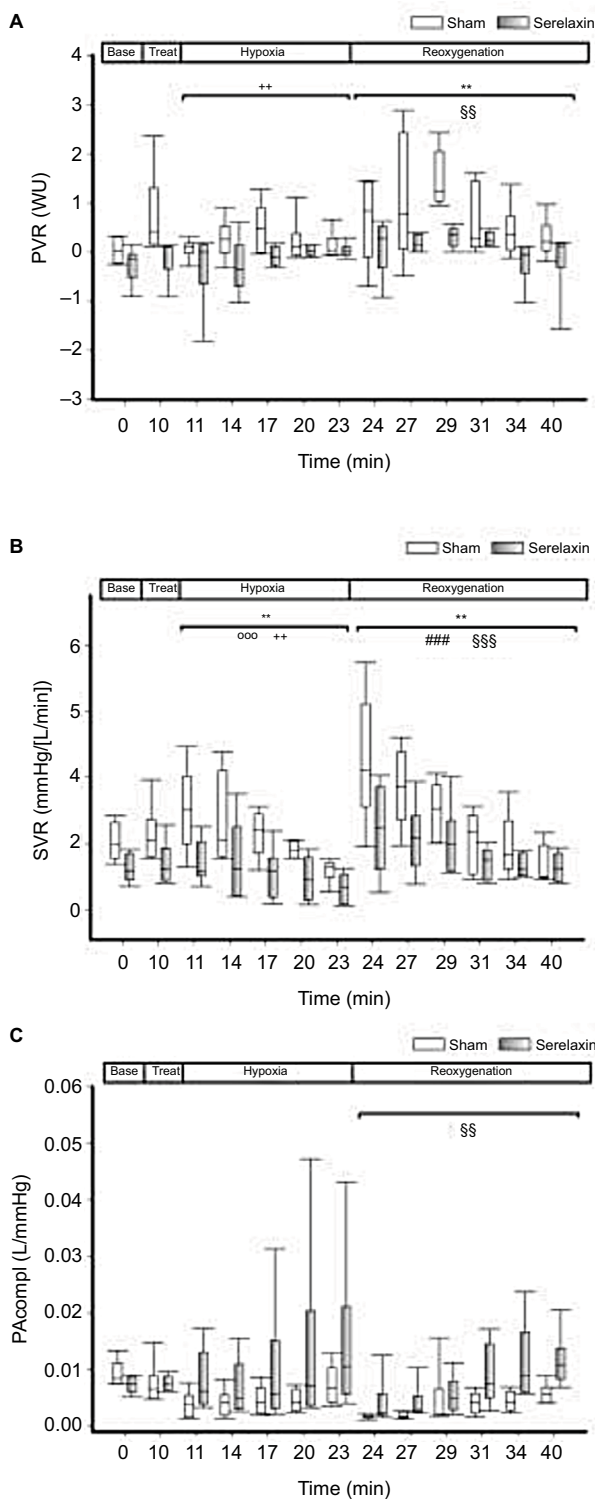
**Notes:** (A) CO and (B) SV.  $^{\circ}$ ,  $p \leq 0.05$ , for comparison of the control group during hypoxia with baseline values.  $+++$ ,  $p \leq 0.001$ , for comparison of the serelaxin-treated group during hypoxia with baseline values.  $###$ ,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the control group.  $$$$$ ,  $p \leq 0.001$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group.

**Abbreviations:** CO, cardiac output; SV, stroke volume.

the detection of low pulmonary hemodynamic responses and furthermore warranted severe physiological response as already successfully applied in preliminary studies.<sup>25</sup> The

applied dosage of 30  $\mu\text{g}/\text{kg}$  serelaxin nonetheless does constitute a rather high dosage of serelaxin. While we decided on utilizing this dose both for the reasons stated above and





**Figure 6** Effects of serelaxin during hypoxia and reoxygenation on pulmonary vascular resistance, systemic vascular resistance, and pulmonary artery compliance. **Notes:** (A) Pulmonary hemodynamic parameters; PVR, (B) SVR, and (C) PAcompl. \*\*,  $p < 0.03$ , for comparison between the two experimental groups during hypoxia or reoxygenation, respectively. °°,  $p < 0.001$ , for comparison of the control group during hypoxia with baseline values. ++,  $p < 0.03$ , for comparison of the serelaxin-treated group during hypoxia with baseline values. ###,  $p < 0.001$ , for comparison of reoxygenation with hypoxia in the control group. §§§,  $p < 0.001$ ; §§,  $p < 0.03$ , for comparison of reoxygenation with hypoxia in the serelaxin-treated group. **Abbreviations:** PVR, pulmonary vascular resistance; SVR, systemic vascular resistance; PAcompl, pulmonary artery compliance; WU, Wood's Unit.

since we already employed similar experimental models in earlier studies, it represents a potential limitation of this study. Our preliminary studies examined relaxin-2 concentrations,<sup>32</sup> relaxin-2 immunoreactivity, and peak relaxin levels, and right hemodynamics after serelaxin administration<sup>26</sup> – a continued comparability and continuity with these earlier results in the context of dosage and its effects on system physiology seemed therefore desirable. However, in a previous large animal model which investigated the cardioprotective properties of relaxin-2, the maximum effective dosage was 5 µg/kg.<sup>50</sup> Since high dosages of relaxin-2 can decrease target organ response due to agonist-induced receptor desensitization,<sup>51</sup> this mechanism was furthermore a likely contributor to the failure of clinical trials with higher dosages. One example of this connection might be a trial on scleroderma patients that utilized dosages of 100 µg/kg,<sup>52</sup> which likely led to a paradoxical disappearance of the desired effect. Therefore, the reported results of this current study might be similarly influenced by the chosen dosage. Future studies should therefore investigate the influence of differing dosages on system physiological alterations and microcirculatory changes of end organs. Most animal and human hypoxic models utilize a 10%–12% oxygen mix, which is already suitable for physiologic response and to observe changes of pulmonary hemodynamics.<sup>2–5,53,54</sup> Our study model, which utilizes a 5% air mix, represents deep hypoxic conditions similar to emergency cases, for example, asphyxia or cardiac arrest.<sup>55,56</sup> We specifically decided on a short observation period of 20 minutes, in order to mimic an emergency medical setting and to explore the validity of serelaxin application in such a setting. Drugs with a short onset of effect (between a few seconds and 30 minutes) are especially needed and preferred in the field of emergency medicine or in medical settings where a hypoxic event is very likely or can be expected to occur. Patients especially at risk of suffering such an event are for example patients suffering from COPD, sleep apnea, or heart failure. Since the half-life period of serelaxin is 6–8 hours, and our experimental setup only allowed for an evaluation of 20 minutes,<sup>48</sup> subsequent studies are needed to assess longer effects of the substance.

MABP and the blood gas values did not differ between both groups over the course of the observation period, which confirms the validity of the experimental model. The elevated hematocrit values (collected from femoral artery) over the course of the observation period can most likely be traced back to small fluid volume loss during the anesthesia.<sup>57,58</sup> An increased HR after a bolus injection of serelaxin has already been described in other studies in humans and sheep.<sup>26,59,60</sup>

This represents another possible limitation of this study, since the systemic effects of our treatment might influence the pulmonary hemodynamics via the Frank–Starling mechanism, which enables beat-to-beat adjustments of the CO in relation to changes of the venous pressure. Nonetheless, an HR-independent examination of hemodynamic parameters is quite difficult, since one of the main effects of relaxin-2 is an increase of HR.<sup>61</sup>

The observed effects of serelaxin on the pulmonary hemodynamics during hypoxia suggest that region-specific control of hemodynamics may be caused by differing relaxin-receptor densities and mechanisms. Earlier studies have noted that the pulmonary vasoconstrictive response to hypoxia is due to a sensor–effector mechanism.<sup>62–65</sup> This sensory mechanism that causes pulmonary vasoconstriction is hypothesized to reside in the mitochondria.<sup>66</sup> The main function of the mitochondria is the generation of adenosine triphosphate (ATP). Electron transport from the electron donors, nicotinamide adenine dinucleotide, and flavin adenine dinucleotide occurs along the electron transport chain. The sensor function of mitochondria is a byproduct of the physiological electron flux.<sup>67,68</sup> Relaxin-2 signaling is very complex and involves multiple pathways.<sup>23,69–71</sup> Experimental studies have demonstrated direct effects of relaxin-2 on mitochondrial function, capacitation, acrosome reaction, adenosine monophosphate (AMP) production, and calcium accumulation.<sup>72,73</sup> The effects on primary AMP and ATP production have been critically discussed.<sup>74,75</sup> Unfortunately, studies investigating the endothelial cells of the pulmonary vascular tree have currently been performed neither in humans nor in sheep. Another possible explanation for the observed changes of pulmonary hemodynamics during hypoxia and reoxygenation is the notch-1 pathway. We speculate that the effects of the pulmonary hemodynamics we observed can either be traced back to mitochondrial signaling or processes controlled by the notch-1 pathway. The aforementioned effects of serelaxin are likely due to interactions with specific plasma membrane receptors. Relaxin-2 activates pathways, whose signaling is mediated via RXFP1 in endothelial cells<sup>69,70,76,77</sup> and which are distributed area-specifically.<sup>78</sup> Improvements of pulmonary hemodynamics are induced through pulmonary vasodilatation via RXFP1- and ET 1-mediation,<sup>35,79</sup> and are effected by decreases of right atrial pressure and SVR.<sup>34,35</sup> The relaxin-2 activated receptors RXFP1 and RXFP2<sup>71</sup> are involved in the remodeling of right heart and pulmonary vessels.<sup>35,79</sup> Studies concerning relaxin-2 signaling and RXFP1 and RXFP2 distribution in sheep have not yet been performed. Further investigations of these densities and signaling functions of the

above-mentioned receptors are needed concerning the sheep animal model. Rapid effects on the pulmonary tree that can be observed after serelaxin administration during hypoxic states are encompassed by activation of phosphoinositide 3-kinase via  $G_{\beta\gamma}$  activation of protein kinase B/Akt, phosphorylation of endothelial NO synthase (eNOS), and the release of NO.<sup>80</sup> Consistent with this hypothesis is that the eNOS/NO pathway has only recently been shown to be crucial for the regulation of PVR.<sup>81</sup>

## Conclusion

The administration of serelaxin prevented hypoxic pulmonary vasoconstriction. Serelaxin might be suitable to improve pulmonary hemodynamics in clinically relevant scenarios during acute hypoxia and reduce the risk of pulmonary hypertension.

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## Author contributions

All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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