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EXPERIMENTAL

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Hypercapnic acidosis transiently weakens hypoxic pulmonary vasoconstriction without affecting endogenous pulmonary nitric oxide production

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Abstract *Purpose:* Hypercapnic acidosis often occurs in critically ill patients and during protective mechanical ventilation; however, the effect of hypercapnic acidosis on endogenous nitric oxide (NO) production and hypoxic pulmonary vasoconstriction (HPV) presents conflicting results. The aim of this study is to test the hypothesis that hypercapnic acidosis augments HPV without changing endogenous NO production in both hyperoxic and hypoxic lung regions in pigs. *Methods:* Sixteen healthy anesthetized pigs were separately ventilated with hypoxic gas to the left lower lobe (LLL) and hyperoxic gas to the rest of the lung. Eight pigs received 10% carbon dioxide (CO_2) inhalation to both lung regions (hypercapnia group), and eight pigs formed the control group. NO concentration in exhaled air (ENO), nitric oxide synthase (NOS) activity, cyclic guanosine monophosphate (cGMP) in lung tissue, and regional pulmonary blood flow were measured. *Results:* There were no differences between the groups for ENO, Ca^{2+}-independent or Ca^{2+}-dependent NOS activity, or cGMP in hypoxic or hyperoxic lung regions. Relative perfusion to LLL (Q_{LLL}/Q_T) was reduced similarly in both groups when LLL hypoxia was induced. During the first 90 min of hypercapnia, Q_{LLL}/Q_T increased from 6% (1%) [mean (standard deviation, SD)] to 9% (2%) ($p < 0.01$), and then decreased to the same level as the control group, where Q_{LLL}/Q_T remained unchanged. Cardiac output increased during hypercapnia ($p < 0.01$), resulting in increased oxygen delivery ($p < 0.01$), despite decreased PaO_2 ($p < 0.01$). *Conclusions:* Hypercapnic acidosis does not potentiate HPV, but rather transiently weakens HPV, and does not affect endogenous NO production in either hypoxic or hyperoxic lung regions.

Keywords Nitric oxide · Hypercapnic acidosis · Hypoxic pulmonary vasoconstriction · Exhaled nitric oxide · Cyclic guanosine monophosphate · Pulmonary blood flow

Introduction

Acute respiratory distress syndrome (ARDS) is associated with both hypoxia and hypercapnic acidosis due to pathological gas exchange in the lung and the use of permissive hypercapnia during mechanical ventilation to prevent pulmonary hyperinflation and ventilator-induced lung injury (VILI) [1, 2]. Hypercapnic acidosis affects the pulmonary and systemic circulation and oxygenation. Hypercapnic acidosis causes pulmonary vasoconstriction

[3, 4], but reports of the effects on hypoxic pulmonary vasoconstriction (HPV) present conflicting results [5–7]. In systemic circulation, hypercapnic acidosis causes vasodilatation through increased NO production [8, 9], but whether hypercapnic acidosis also causes changes in endogenous NO production in the pulmonary circulation is unclear. NO is an important regulator of pulmonary blood flow, and blockade of enzymatic NO production enhances HPV [10, 11]. In ARDS patients, HPV can contribute up to 20 torr to arterial oxygenation [12]. If hypercapnic acidosis increases NO production in hypoxic lung regions, this would attenuate HPV, and could be detrimental for patients suffering from severe ARDS.

Metabolic acidosis augments HPV, without any change in endogenous pulmonary NO production [13]; therefore, it was hypothesized that hypercapnic acidosis has similar effects. The aim of this study is to test the hypothesis that hypercapnic acidosis augments HPV without any changes in pulmonary NO production in both hyperoxic and hypoxic lung regions in pigs.

Materials and methods

The study was approved by the Animal Research Ethics Committee of Uppsala University, Uppsala, Sweden. Sixteen healthy pigs (Swedish country breed, weight 25–30 kg) were premedicated with intramuscular injection of 6 mg/kg Soletil Forte (Tiletamin and Zolazepam) and 2.2 mg/kg Rompun (Xylazin chloride) before anesthesia induction with 40–100 mg propofol given intravenously. Anesthesia was maintained with propofol infusion at rate of 3 mg/kg/h and infusion of ketamin vet. (5 g), fentanyl (1 mg), and pancuronium (60 mg) in 1,000 ml buffered glucose (25 mg/ml) at rate of 4 ml/kg/h. Oxygen saturation, and inspiratory and end-tidal concentrations (C_{ET}) of oxygen (O_2) and CO_2 were monitored continuously (Datex AS/3^{TM} anesthesia monitor; Datex Ohmeda, Helsinki, Finland) during the whole experiment. Warm buffered Ringer's solution (10–15 ml/kg/h) was infused, and a suprapubic catheter was inserted for urinary output. Throughout the experiment, the pigs were laid supine on a heating mattress with warm blankets to maintain normal and stable body temperature.

Ventilation

A tracheotomy was performed, and a cuffed endotracheal tube (inner diameter 6.0 mm) was inserted. A second cuffed endotracheal tube (inner diameter 4.5 mm) was inserted through the tracheostoma and positioned in the left lower lobar bronchus. A medial sternotomy allowed the tubes to be guided into a position to separate the left lower lobe (LLL) from the other lung regions, which meant the lungs could be inspected to ensure the left middle and upper lobes and the right lung were ventilated through the main tube. The different and persistent fractions of expired O_2 during LLL hypoxia and hyperoxia to the other lung regions were considered additional proof of separation. The lungs were mechanically ventilated by two synchronized Servo 900 C ventilators (Siemens Elema, Lund, Sweden). Both ventilators were set at volume-controlled ventilation of 20 breaths per minute and an inspiration-to-expiration ratio of 1:2. Positive end-expiratory pressure (PEEP) of 5 cmH_2O was applied, and a total tidal volume of 8 ml/kg was distributed between the LLL and the other lung regions, aiming at equal end-inspiratory plateau pressures in the LLL and other parts of the lungs. The minute ventilation was then adjusted, if needed, by increasing the respiratory rate to obtain arterial CO_2 tension ($PaCO_2$) of 41–49 mmHg (5.5–6.5 kPa), and a corresponding normal pH of 7.35–7.45 in the initial control situation. The ventilation was then kept constant throughout the experiment.

Hemodynamics

An arterial catheter, a central venous catheter, and a pulmonary artery catheter were inserted (Criti Cath^{TM} No 7F; Ohmeda Pte Ltd, Singapore) to record arterial, central venous, and pulmonary blood pressures and temperatures (Datex AS/3^{TM} anesthesia monitor; Datex Ohmeda, Helsinki, Finland). Cardiac output (Q_T) and blood flow to the LLL (Q_{LLL}) were measured continuously by enclosing the pulmonary artery and the artery to the LLL in ultrasonic flow probes connected to flow meters (T208 Transonic volume flow meter; Transonic Systems Inc., Ithaca, NY, USA). The relative perfusion of the LLL was calculated as Q_{LLL}/Q_T.

Blood gases

Mixed venous and arterial blood samples were collected for analysis of O_2 tensions (PvO_2 and PaO_2), $PaCO_2$, and pH (ABL 625; Radiometer, Copenhagen, Denmark), and arterial O_2 saturation (SaO_2) and methemoglobin (MetHb) (OSM 3; Radiometer, Copenhagen, Denmark).

Exhaled nitric oxide concentration (ENO)

ENO was measured alternately from the hypoxic LLL and the hyperoxic lung regions by chemiluminescence (analyzer model 42; Thermo Environmental Instruments Inc., Franklin, MA, USA). The measurements were taken in the expiratory limb of the ventilator tubings and more than 100 cm from the endotracheal tubes, which ensured complete mixing and avoided contamination by inspired gas. The average concentration (mean expired values) over ten breaths was used for the statistical analyses.

Nitric oxide synthase activity (NOS)

NOS activity was measured by standard procedure as presented in detail elsewhere [13].

cGMP enzyme immunoassay (EIA)

For quantification of cGMP, lung tissue lysates were analyzed with a commercially available EIA kit (Detect X® Direct Cyclic GMP; Arbor Assays, MI, USA), and according to manufacturer's instructions. Optical density was read at 450 nm, which was corrected at 570 nm with a Tecan Sunrise instrument (Tecan Nordic AB, Mölndal, Sweden) with Magellan software. For normalization, the protein concentration in the tissue lysates was determined with a commercially available protein assay kit based on the Bradford assay (Coomassie Plus Assay Kit; Thermo Scientific, IL, USA).

Experimental protocol

Ventilatory and hemodynamic parameters were measured and blood was sampled for mixed venous and arterial blood gases 30 min after preparation and ventilation with hyperoxic gas [fraction of inspired O_2 (F_IO_2) 0.8, balance nitrogen] to both lungs (baseline). The inhaled gas was then changed from hyperoxic to hypoxic (F_IO_2 0.05, balance nitrogen) to the LLL, and data were collected after 30 min of regional LLL hypoxia. The pigs were then randomized into the control group ($n = 8$) or hypercapnia group ($n = 8$). In the control group, regional LLL hypoxia was continued throughout the experiment. In the hypercapnia group, the LLL was ventilated with 10% CO_2 in 5% O_2, balance nitrogen; the rest of the lung was ventilated with 10% CO_2 in 80% O_2, balance nitrogen throughout the experiment. Data were collected every 30 min during 3.5 h in both groups.

ENO was measured alternately from the hypoxic LLL and the hyperoxic lung regions (HL). Blood flow in the main pulmonary artery (Q_T) and Q_{LLL} were measured continuously. At the end of each experiment, with the pig anesthetized, alive, and ventilated, pieces from the hypoxic LLL and HL were excised and immediately frozen in liquid nitrogen for analysis of NOS activity and cGMP. Finally, the pigs were euthanized with an intravenous injection of potassium chloride (KCl).

Statistical analyses

Data in the text and tables are presented as mean (SD). A two-way analysis of variance for repeated measures (ANOVA) on one factor was applied to disclose any interaction effects (pAB) or differences within or between groups (pA). Data collection periods (fixed) and pigs (random) were the two block factors for comparisons within the groups. Groups (fixed) and pigs (random) were the two block factors for comparisons between the groups. Tukey's test was used as the post hoc test. A probability of <0.05 was accepted as significant. A one-way ANOVA was used for analysis of NOS activity and cGMP. All analyses were performed with Statistica (version 8; Statsoft Inc., Tulsa, OK, USA).

Results

Thirty minutes of CO_2 inhalation (hypercapnia group) decreased pH from 7.38 (0.03) to 7.10 (0.02) ($p < 0.01$); the pH then slowly decreased to 7.01 (0.03). pH was normal in the control group throughout the experiment [7.40 (0.03)–7.37 (0.03)] (Table 1).

ENO and NOS activity

There were no differences between the groups for ENO from the hypoxic LLL or from the hyperoxic lung regions (Table 1), nor were there any differences between the hypercapnia and control groups for Ca^{2+}-independent (iNOS) or Ca^{2+}-dependent (cNOS) activity in hypoxic or hyperoxic lung regions (Fig. 1). In the hyperoxic lung, cGMP was 8.2 (6.5) pmol/mg in the control group and 4.1 (1.6) pmol/mg in the hypercapnia group ($p = 0.1$), and in hypoxic lung, cGMP was 3.7 (1.5) pmol/mg in the control group and 2.6 (1.4) pmol/mg in the hypercapnia group ($p = 0.2$).

Pulmonary hemodynamics

Q_{LLL}/Q_T decreased by 72% (5%) during LLL hypoxia in the hypercapnia group and by 68% (9%) in the control group; there was no further change in Q_{LLL}/Q_T throughout the experiment in the control group. Inhalation of CO_2 increased Q_{LLL}/Q_T ($p < 0.01$) from 6% (1%) to 9% (2%). Q_{LLL}/Q_T remained elevated for 1.5 h in the hypercapnia group and then declined to reach the same level as in the control group after 3.5 h (Fig. 2a). Mean pulmonary arterial pressure (MPaP) increased in the hypercapnia group, compared with the control group ($p < 0.01$). There were no differences between the groups for PVR, PVR_{LLL}, or PVR_{HL} (Table 2). The pulmonary arterial diastolic-pulmonary capillary wedge pressure (Ppadiast–PcwP) gradient increased after 90 min of CO_2 inhalation in the hypercapnia group ($p < 0.01$) (Table 2).

Table 1 Blood gases and exhaled nitric oxide

	Baseline hyperoxia	30 min LLL hypoxia	60 min LLL hypoxia	90 min LLL hypoxia	120 min LLL hypoxia	150 min LLL hypoxia	180 min LLL hypoxia	210 min LLL hypoxia	240 min LLL hypoxia	P^{AB}
pH										
Control	7.40 (0.03)	7.38 (0.04)	7.39 (0.03)	7.39 (0.03)	7.38 (0.03)	7.39 (0.03)	7.38 (0.03)	7.38 (0.03)	7.37 (0.03)	<0.01
Hypercapnia	7.40 (0.03)	7.38 (0.03)	7.10 (0.02)	7.06 (0.05)	7.05 (0.05)	7.04 (0.05)	7.02 (0.04)	7.01 (0.04)	7.01 (0.04)	
$PaCO_2$ (torr)										
Control	48 (4)	52 (5)	51 (4)	52 (4)	52 (4)	51 (4)	53 (4)	52 (4)	54 (8)	<0.01
Hypercapnia	50 (5)	51 (4)	111 (7)	119 (6)	124 (8)	128 (9)	131 (11)	135 (14)	134 (9)	
ENO_{LLL} (ppb)										
Control	5.4 (5.1)	6.6 (6.6)	6.3 (5.5)	7.6 (7.4)	8.1 (7.5)	7.4 (5.6)	7.3 (6.6)	7.6 (6.3)	6.7 (5)	0.99
Hypercapnia	7.0 (5.5)	8.6 (8.7)	8.3 (8.1)	8.9 (8.1)	9.8 (10.1)	9.7 (10.6)	9.2 (7.5)	8.4 (8.7)	7.2 (6.1)	
ENO_{HL} (ppb)										
Control	5.5 (4.3)	7.0 (7.0)	6.7 (5.4)	7.6 (7.7)	8.1 (7.7)	7.3 (5.6)	7.2 (6.6)	7.2 (6.2)	6.4 (4.5)	1.0
Hypercapnia	7.4 (6.5)	8.5 (8.8)	7.8 (7.5)	9.5 (9.5)	9.7 (10.4)	9.1 (9.4)	8.5 (7.3)	8.3 (8.7)	7.0 (6.3)	
PvO_2 (torr)										
Control	40 (6)	39 (5)	39 (4)	38 (4)	39 (5)	39 (5)	38 (5)	39 (5)	38 (4)	< 0.01
Hypercapnia	54 (34)	41 (4)	53 (5)	56(4)	59 (5)	60 (5)	60 (5)	60 (6)	59 (6)	

Mean (SD) $n = 8$

$PaCO_2$ partial pressure of carbon dioxide in arterial blood, *ENO* nitric oxide concentration in exhaled gas, *ppb* parts per billion, *LLL* left lower lobe, *HL* hyperoxic parts of the lungs, *PvO_2* partial pressure of oxygen in mixed venous blood, *P^{AB}* interaction effect of hypercapnia over time

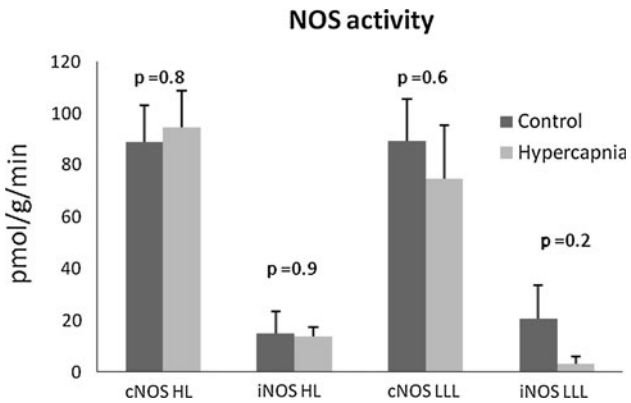

Fig. 1 Nitric oxide synthase activity in lung tissue (pmol g^{-1} min^{-1}). Mean (standard error of the mean, SEM). There were no significant differences in the Ca^{2+}-dependent or Ca^{2+}-independent NOS activities between the groups

Blood gases and oxygen delivery

PaO_2 decreased in both groups with LLL hypoxia. A further decrease was observed in the hypercapnia group ($p < 0.01$), but PaO_2 remained unchanged in the control group (Fig. 2b). Oxygen delivery and PvO_2 increased ($p < 0.01$) in the hypercapnia group, compared with the control group (Tables 1, 2).

Systemic hemodynamics

In the hypercapnia group, Q_T increased after introduction of CO_2 inhalation ($p < 0.01$) and remained elevated throughout the experiment, whereas Q_T did not change over time in the control group (Fig. 2c). Central venous pressure (CVP) and PcwP did not differ between the groups, but heart rate (HR) increased ($p < 0.01$) and systemic vascular resistance (SVR) decreased ($p < 0.01$) in the hypercapnia group (Table 2). The intrapulmonary shunt (Q_S/Q_T) increased similarly in both groups with LLL hypoxia. A further increase was observed in the hypercapnia group ($p < 0.01$), whereas Q_S/Q_T remained unchanged in the control group (Table 2).

Discussion

The major finding in the present study was that hypercapnic acidosis did not affect endogenous NO production, in either hypoxic or hyperoxic lung regions, as indicated by the lack of changes in ENO, NOS activity, and cGMP. Hypercapnic acidosis did not potentiate HPV during the time span of the study. However, a transient weakening of HPV was observed.

Hypercapnic acidosis and endogenous NO production

ENO measured from a tracheostoma, as in this study, reflects NO production from the lower airways, providing that other factors that influence ENO, such as changes in ventilation, PEEP, F_IO_2, and total and regional blood flow [14], remain constant. To eliminate any influence on the results, CO_2 was added to the inspiratory gas mixture to induce hypercapnic acidosis, and the ventilator settings and PEEP were kept constant.

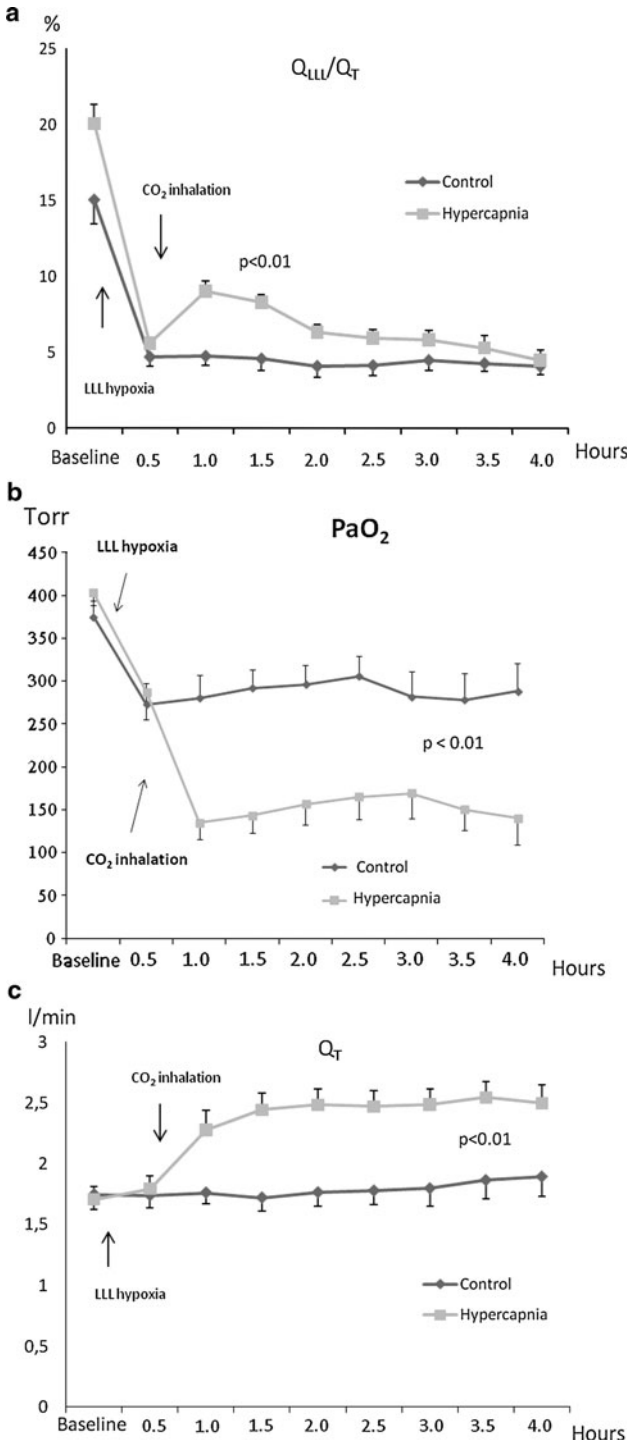

Fig. 2 **a**, **b**, **c** Mean (SEM). p values = significant interaction effect (pAB) of hypercapnia over time between the control group ($n = 8$) and the hypercapnia group ($n = 8$). **a** Q_{LLL}/Q_T. Blood flow to the LLL (Q_{LLL}) in relation to cardiac output (Q_T). **b** PaO_2 (partial pressure of oxygen in arterial blood). **c** Cardiac output (Q_T)

Q_T increased during hypercapnia. An increased pulmonary blood flow increases shear stress, stimulates endothelial NOS (eNOS) activity, and increases ENO [15]. ENO can also be expected to decrease due to increased pulmonary blood flow, as more NO is scavenged by the blood and less escapes into the exhaled air [14]. In the present study, no changes in ENO from either hypoxic or hyperoxic lung regions were observed during hypercapnic acidosis.

Severe hypoxia, not compatible with survival, decreases ENO, which is consistent with NO synthesis from L-arginine requiring molecular oxygen [14, 16, 17]. Moderate hypoxia, compatible with life, does not affect, or increases ENO [16–19]. Increased NO production during hypoxia is proposed [20, 21] as an intrinsic system for protecting the individual from injurious pulmonary hypertension and risk of right heart failure.

iNOS activity was generally lower than cNOS activity in both groups, which concurred with findings from previous studies in healthy and endotoxemic pigs [22, 23]. Enzymes operate at an optimal intracellular pH, which can differ depending on cell type. Endothelial NOS activity increases during alkalosis via influx of extracellular calcium and decreases during acidosis [24]; in cultured cells [24, 25], alkalosis favors cNOS activity and acidosis favors iNOS activity. However, we could not demonstrate that hypercapnic acidosis caused any statistically significant changes in cNOS or iNOS activity in either hypoxic or hyperoxic lung regions in pigs.

Hypercapnic acidosis and HPV

Hypercapnic acidosis increased both Q_T and Q_{LLL}/Q_T during the first 90 min; HPV was transiently attenuated. There are several mechanisms related to hypercapnia-induced Q_T increase: first, hypercapnia can initiate a sympathetically mediated release of catecholamines due to neuroadrenal stimulation [26, 27]; second, hypercapnic acidosis induces adenosine triphosphate (ATP)-sensitive K^+ channel-mediated vasodilatation [28]; and, third, preload may be increased via venoconstriction in acidemia [26]. Therefore, Q_T may increase because of increased preload, decreased afterload, and increased contractility. Increased heart rate and decreased afterload were the most obvious mechanisms observed in this study.

Intrapulmonary shunt (Q_S/Q_T) varies directly with Q_T. An increased Q_T decreases HPV due to a combination of increases in PvO_2 [29, 30], pulmonary artery pressure [31], and pulmonary blood flow [32, 33]. All these factors probably contributed to the transient decrease in HPV. The increase in Q_T and PvO_2 persisted, whereas Q_{LLL}/Q_T slowly declined to values similar to the control group. Simultaneous with the transient increase in Q_{LLL}, a decrease in PVR_{LLL} was observed (Table 2), but this decrease did not reach statistical significance ($p = 0.07$). PVR_{HL} did not increase in the hypercapnic pigs, indicating that the transient redistribution of pulmonary blood

Table 2 Hemodynamic variables

	Baseline hyperoxia	30 min LLL hypoxia	60 min LLL hypoxia	90 min LLL hypoxia	120 min LLL hypoxia	150 min LLL hypoxia	180 min LLL hypoxia	210 min LLL hypoxia	240 min LLL hypoxia	P^{AB}
MPaP (mmHg)										
Control	22 (3)	25 (2)	25 (2)	25 (1)	26 (1)	25 (1)	26 (2)	27 (3)	26 (1)	<0.01
Hypercapnia	20 (2)	23 (3)	28 (4)	30 (4)	31 (4)	31 (4)	31 (3)	31 (4)	31 (3)	
PVR (dyn/s)										
Control	650 (140)	740 (150)	780 (120)	820 (100)	790 (80)	750 (110)	790 (250)	830 (290)	790 (240)	0.8
Hypercapnia	630 (130)	700 (180)	720 (250)	760 (230)	730 (180)	750 (180)	760 (210)	690 (190)	710 (200)	
PVR_{LLL} (dyn/s)										
Control	4,400 (980)	17,600 (7,960)	18,260 (7,275)	21,090 (11,040)	24,780 (18,360)	24,010 (18,340)	18,890 (8,830)	19,000 (7,790)	19,970 (8,700)	0.07
Hypercapnia	3,200 (940)	12,600 (2,800)	8,450 (4,340)	9,430 (3,060)	11,960 (3,150)	13,720 (4,380)	14,080 (5,050)	14,430 (3,820)	15,600 (3,560)	
PVR_{HL} (dyn/s)										
Control	770 (200)	780 (150)	820 (130)	860 (120)	820 (90)	780 (130)	830 (270)	870 (300)	820 (250)	0.8
Hypercapnia	790 (160)	740 (190)	790 (270)	830 (250)	780 (200)	800 (190)	810 (220)	750 (230)	740 (230)	
Ppadiast–PcwP gradient										
Control	7 (3)	8 (2)	9 (2)	9 (1)	9 (2)	9 (3)	10 (3)	10 (5)	9 (3)	<0.01
Hypercapnia	6 (2)	8 (3)	10 (4)	12 (3)	13 (2)	14 (2)	16 (3)	14 (6)	13 (4)	
MaP (mmHg)										
Control	67 (9)	70 (8)	70 (8)	70 (9)	71 (8)	71 (8)	72 (9)	70 (8)	70 (8)	0.05
Hypercapnia	77 (11)	75 (6)	78 (6)	80 (5)	82 (6)	82 (7)	82 (6)	84 (6)	81 (7)	
CVP (mmHg)										
Control	8 (1)	8 (2)	8 (1)	9 (1)	9 (2)	9 (2)	9 (1)	9 (1)	9 (1)	0.4
Hypercapnia	7 (1)	7 (1)	8 (1)	8 (1)	8 (1)	8 (1)	8 (1)	8 (1)	8 (2)	
PcwP (mmHg)										
Control	8 (1)	9 (2)	8 (2)	8 (1)	9 (2)	9 (2)	9 (2)	9 (2)	9 (2)	0.5
Hypercapnia	7 (1)	8 (1)	9 (1)	8 (2)	9 (2)	8 (2)	8 (2)	9 (2)	9 (1)	
HR										
Control	83 (9)	85 (9)	88 (10)	88 (10)	91 (10)	93 (10)	95 (9)	97 (12)	99 (13)	<0.01
Hypercapnia	83 (7)	87 (12)	101 (16)	112 (16)	118 (15)	121 (15)	123 (16)	125 (19)	123 (16)	
SVR (dyn/s)										
Control	2,760 (560)	2,880 (500)	2,850 (440)	2,880 (520)	2,940 (530)	2,840 (480)	2,880 (560)	2,710 (540)	2,680 (620)	<0.01
Hypercapnia	3,390 (770)	3,130 (570)	2,550 (540)	2,420 (420)	2,420 (330)	2,400 (330)	2,420 (330)	2,450 (350)	2,370 (470)	
Q_S/Q_T (%)										
Control	21 (8)	36 (11)	36 (12)	33 (9)	33 (9)	32 (10)	34 (12)	35 (13)	34 (15)	<0.01
Hypercapnia	17 (7)	37 (16)	65 (9)	65 (10)	64 (10)	61 (12)	61 (14)	63 (13)	60 (13)	
DO_2 (ml/min)										
Control	214 (41)	205 (36)	207 (26)	202 (35)	201 (33)	207 (34)	205 (39)	200 (41)	199 (35)	<0.01
Hypercapnia	212 (44)	211 (36)	264 (50)	289 (42)	302 (41)	301 (42)	304 (43)	310 (45)	302 (44)	

Mean (SD) $n = 8$

MPaP mean pulmonary arterial pressure, *PVR* pulmonary vascular resistance, *LLL* left lower lobe, *HL* hyperoxic parts of the lungs, *Ppadiast–PcwP gradient* pulmonary arterial end diastolic–pulmonary wedge pressure gradient, *MaP* mean systemic arterial pressure, *CVP* central venous pressure, *PcwP* pulmonary capillary wedge pressure, *HR* heart rate, *SVR* systemic vascular resistance, Q_S/Q_T pulmonary shunt, DO_2 delivery of oxygen. p^{AB} interaction effect of hypercapnia over time

flow to the LLL could not be explained by higher impedance in the hyperoxic lung regions.

Acidosis causes dilatation in the systemic vasculature and constriction in the pulmonary vasculature, because pH differentially regulates voltage-gated potassium channels in pulmonary and systemic vascular smooth muscle cells, thus modulating vascular reactivity [34]. Hypercapnic acidosis has two components: the CO_2 molecule and the resulting activity of hydrogen ions (pH).

The vasoactive action of CO_2 is dependent on the initial PVR. During basal tone condition, CO_2 is a mild vasoconstrictor, whereas, at high PVR, such as in hypoxia, it is a potent vasodilator [5, 35–37]. The effect of CO_2 dilatation is proposed to have a direct action on smooth muscle, and constriction is caused by decreasing pH [37]. Hence, depending on the balance between the dilating effect of the CO_2 molecule and the vasoconstrictive effect of the hydrogen ion, hypercapnia can be expected to both attenuate and augment HPV.

The observed transient decrease in HPV was attributed to both the vasodilating effect of the CO_2 molecule and the increase in Q_T. This effect was mainly caused by hypercapnia, as acidosis per se augments HPV. However, as the intracellular pH decreased, the vascular tone in the hypoxic lung regions increased and HPV returned to initial values. Extracellular pH decreased rapidly to 7.10, and then slowly to pH 7.01. The rate of change in intracellular pH might differ from the rate of change in extracellular pH, as intracellular pH in the pulmonary capillary bed is regulated by membrane-bound carbonic anhydrase [38]; CO_2 freely crosses cell membranes, whereas hydrogen ions do not.

The effects of hypercapnic acidosis on HPV vary depending on species and experimental models [5, 6, 39]. The use of isolated lungs perfused with blood-free solution and constant Q_T [6, 7, 40] or the use of intact animals will yield different results. The perfused lung is denervated and isolated from the systemic circulation, with particular concerns being related to the lack of pulmonary–systemic interaction. The intact large animal model has the advantage of more closely resembling the clinical situation and physiology in patients, although data in animals cannot readily be extrapolated to humans.

There was no change in PVR in the hypercapnic pigs in this study; however, the calculation of PVR becomes difficult to interpret as Q_T increases. Therefore, the Ppadiast–PcwP gradient may be more accurate for expressing resistance to flow through the pulmonary vascular bed [41]. The Ppadiast–PcwP gradient increased in the hypercapnia group after 90 min of CO_2 inhalation, which indicated an increased pulmonary vascular tone. Thus, pulmonary hypertension in the hypercapnic pigs could be explained by increased pulmonary blood flow and increasing pulmonary vascular tone over time.

Hypercapnic acidosis and oxygenation

Hypercapnic acidosis decreased PaO_2. The hypercapnia-induced increase in Q_T and intravascular pulmonary pressure combined with a vasodilatory effect of CO_2 resulted in recruitment of pulmonary vessels in poorly ventilated lung regions and led to an increase in Q_S/Q_T. A decreased alveolar partial pressure of oxygen (P_AO_2), due to the increase in inspiratory CO_2 partial pressure, was considered an additional explanation for the decrease in PaO_2. However, as the calculation of P_AO_2 revealed no differences between the groups, it was reasonable to conclude that the decrease in PaO_2 was explained by increased shunt and ventilation/perfusion (V/Q) mismatch. Although PaO_2 decreased, the net effect of hypercapnic acidosis on tissue oxygenation was increased oxygen delivery caused by increased Q_T.

Limitations

In this study, the pigs were healthy, which is contrary to the clinical situation, where hypercapnic acidosis typically occurs in hemodynamically and respiratory compromised critically ill patients, where particular concerns are related to oxidative stress, iNOS induction, inflammatory mediators, etc. Hypercapnic acidosis was induced by inhalation of CO_2, instead of permissive hypercapnia mimicking the clinical situation. However, any changes in the ventilator settings would have affected ENO, thus rendering it impossible to evaluate the effects of hypercapnic acidosis on endogenous pulmonary NO production, as was the aim of the study. If pH reduction is the same, similar results would be expected irrespective of whether hypercapnia was induced through low minute ventilation or CO_2 inhalation. The study design was considered appropriate for eliminating as many confounding factors as possible and standardizing the level of hypercapnic acidosis throughout the lung.

Conclusions

Hypercapnic acidosis did not potentiate HPV; instead, a transient weakening of HPV was observed. This effect was mainly caused by hypercapnia, as acidosis per se augments HPV. Hypercapnic acidosis did not affect endogenous NO production in either hypoxic or hyperoxic lung regions, as indicated by the lack of changes in ENO, NOS activity, or cGMP. Cardiac output increased during hypercapnic acidosis, resulting in increased oxygen delivery. At the same time, PaO_2 decreased due to increased shunt and/or V/Q mismatch.

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