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Development of a piglet model for cerebrovascular autoregulation assessment with altered $PaCO_2$

Sofie Dietvorst [a,b,*], Veerle Desloovere [c,d], Geert Meyfroidt [d,e], Bart Depreitere [a,b]

[a] Department of Neurosurgery, University Hospitals Leuven, Belgium
[b] Research Group Experimental Neurosurgery and Neuroanatomy, KULeuven, Belgium
[c] Department of Anesthesiology, University Hospitals Leuven, Belgium
[d] Laboratory of Intensive Care Medicine, KULeuven, Belgium
[e] Department of Intensive Care Medicine, University Hospitals Leuven, Belgium

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ABSTRACT

Introduction: Cerebrovascular autoregulation (CA) capacity can be impaired in the aftermath of acute brain injuries. Altered physiological states, such as hypo- and hypercapnia, affect CA. Although these effects have been demonstrated in several animal experiments, the exact effect of $PaCO_2$ on the plateau of cerebral blood flow (CBF) across the spectrum of arterial blood pressures has not been fully disclosed.
Research question: The aim was to explore pial vasodynamics in response to changing $PaCO_2$ in a porcine cranial window model, as preparation for an experimental setup in which the CBF plateau position is investigated under different $PaCO_2$ conditions.
Material and methods: Five piglets were brought under anesthesia, intubated, ventilated and instrumented with a cranial window through which pial arteriolar diameters could be microscopically observed. By changing ventilation to either hyper- or hypoventilation we were able to investigate a range of PaCO2 from 25 till 90 mmHg.
Results: Altering the respiratory rate to manipulate $PaCO_2$ by ventilation appeared to be feasible and reliable.
Discussion and conclusion: We found that $ETCO_2$ reliably represents $PaCO_2$ in our model. Pial arteriolar diameter changes followed the direction of $PaCO_2$ changes, but the effect of $PaCO_2$ on the diameters was not linear. Only in the hypercapnia setting did we observe a clear and consistent vasodilation of the pial arterioles.

1. Introduction

Carbon dioxide (CO_2) is a potent vasodilator in cerebral hemodynamics. However, not CO_2 itself, but the change of extracellular pH in the interstitial fluid secondary to CO_2, has been shown to be the main driver affecting cerebrovascular resistance (CVR). It was proven that perivascular pH is inversely related to pial arterial diameter (Muizelaar et al., 1988; Madden, 1993; Yoon et al., 2012). This was confirmed in experiments with tromethamine (THAM), an alkaline buffer that does not affect CO_2, but does lower intracranial pressure (ICP) in acute neurological injury (Zeiler et al., 2014; Muizelaar et al., 1991; Wolf et al., 1993). CO_2 can have an effect on CVR during the transient shift in interstitial pH. This effect lessens once metabolic compensation for the pH change caused by CO_2 fluctuations has occurred, which has been shown to take about 12–24 h (Berend et al., 2014; Brian, 1998). However, because CO_2 is lipophilic and can cross the blood-brain barrier (BBB), whereas loaded H+ ions cannot, respiratory acidosis has a greater influence on CVR than metabolic acidosis. As a result, arterial carbon dioxide partial pressure ($PaCO_2$) is the main determinant of interstitial pH(Muizelaar et al., 1988; Kontos et al., 1977). Several feedback loops counteract elevated $PaCO_2$ and its effects. Due to vasodilation, the subsequent increase in cerebral blood flow (CBF) leads to a washout of CO_2. Further, the central respiratory chemoreflex (situated in the ventral medulla oblongata) responds to increases of $PaCO_2$ by increasing ventilation frequency and/or depth. Conversely, the peripheral respiratory chemoreflex initiated by the carotid and aortic sinuses acts upon decreases in PaO_2 which occurs when it drops below 50 mmHg (Ogoh, 2019; Schaeffer and Iadecola, 2021). Partial pressures of CO_2 in the arterial blood and in the cerebrospinal fluid measured in the intracranial cisterns have similar values (Ogoh, 2019). However, they are not

* Corresponding author. Department of Neurosurgery, University Hospitals Leuven, Belgium.
E-mail address: sofie.dietvorst@alderhey.nhs.uk (S. Dietvorst).

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similar to those in lumbar cerebrospinal fluid since the response to CO_2 changes here is delayed due to distance (Andrews et al., 1994). Changing $PaCO_2$ also influences the Hemoglobin (Hb) dissociation curve through the change in arterial pH. This curve depicts the relation between PaO_2 (in mmHg) and Oxyhemoglobin (saturation in %). Decreasing pH shifts the curve to the right, so the Hb affinity for O_2 decreases. This helps deliver O_2 to tissues and is called the Bohr effect (Benner et al., 2022; Bö et al., 2020).

In spite of the mentioned experimental physiological knowledge, the exact effect of $PaCO_2$ on the height and width of the plateau of CBF across the spectrum of arterial blood pressures (ABP) in large animals nor humans has neither been fully disclosed nor quantified. In particular, Gelb et al. published a review of the available research on the impact of hypo- and hypercapnia on cerebrovascular autoregulation (CA), where they concluded that several hypotheses exist for the relation of hypocapnia and the upper limit of autoregulation (Meng and Gelb, 2015).

Our lab(Klein et al., 2019) has been able to revisit the autoregulation curve, first introduced by Lassen, by applying mechanically induced blood pressure changes in a modified porcine cranial window model which allowed to record both changes in pial arteriolar diameter and red blood cell velocity (Klein et al., 2019; Lassen, 1959). Pigs, as opposed to other animal models, have a brain morphology and physiology (including BBB and CA) and a cardiopulmonary physiology close to humans (Duhaime, 2006). We want to investigate what the direct influence of different levels of $PaCO_2$ is on pial diameters, to confirm if hypercapnia induces vasodilation and hypocapnia induces vasoconstriction of pial arterioles in our model.

2. Materials and methods

2.1. Ethical considerations

All animal care and procedures were approved by the Ethics Committee Animal Research Center, KULeuven (Ethical Approval: P107-2019) in compliance with the Belgian Royal Decree (May 29, 2013) and European Directive 2010/63/EU on the protection for animals used for scientific purposes. All animal procedures were conducted under veterinarian supervision according to the guidelines imposed by the Ethical Committee.

2.2. Experimental setup

To alter $PaCO_2$ in sedated and ventilated animals, there are two possibilities. The first is to blend CO_2 in the inhaled mixture of air and O_2. This is probably the easiest method and does not influence the ventilation settings. The second is adjusting the ventilator to hyper- or hypoventilation and hereby influencing the level of $PaCO_2$. However, this might also influence the intrathoracic pressure and thus alters also the basic cardiopulmonary physiology (Stocchetti et al., 2005). We decided to alter ventilation, since this seems the most comparable to human data. Hyper- and hypoventilation are not only common in diseases but are also used as a therapy. We kept the tidal volume (TV) constant but changed the respiration rate (RR), since it has been shown that mechanical hyperventilation with low RR and large TV generally reduces the blood flow to tissues. By increasing RR with a constant TV, there are no changes in cardiac output (CO) expected, which is important in our model where we want to strive for a normal physiological setting (Stocchetti et al., 2005; KARLSSON et al., 1994).

For this exploratory study, $PaCO_2$ was manipulated from the lowest achievable $PaCO_2$ possible to the highest achievable or vice versa, where the animals remained hemodynamically stable. Experiments were based on the porcine cranial window model described by Klein et al. (2019) Five 6-week-old male piglets (domestic swine, Zootechnical Center KU Leuven) were brought under general anesthesia by intravenous propofol, pancuronium, midazolam and fentanyl, intubated and ventilated. An arterial line was placed in the left femoral artery for continuous ABP monitoring, placed 5 cm above the diaphragm. Subsequently, we made two small cranial burr holes posterior to the coronal suture, on the right side, one for ICP with brain tissue oxygen monitoring (PbO_2) (Neurovent-PTO, Raumedic AG, Muenchenberg Germany) and laser Doppler flow (LDF) (Moor VMS-LDF1 with VP14–CBF probe, Moor Instruments, Devon UK) monitoring. Anterior to the coronal suture, a round craniotomy was performed for placement of the cranial window, which was cemented to the skull.

2.3. Monitoring

ABP, ICP, PbO_2, brain temperature, LDF and heart rate (3 lead ECG and arterial pulse wave analysis) were continuously monitored (Philips Intellivue monitor, Philips Medical Systems, the Netherlands). Cerebral perfusion pressure (CPP) is calculated as ABP minus ICP. Blood oxygen level was measured with a pulse oximeter and kept above 95%. Inspired and expired concentrations of CO_2 and O_2 were measured with a gas analyzer (Phillips M1026B, Philips Medical Systems, The Netherlands). Rectal temperature was kept at normothermia of 38–39 °C by warming mattress and blankets. The ICM + software (Cambridge University, Cambridge, United Kingdom, https://icmplus.neurosurg.cam.ac.uk/) was used to integrate and record all the monitoring data. ABP, ICP and LDF signals were sampled real-time at 250Hz.

2.4. Cranial window in vivo imaging

Pial arterioles were observed through the cranial window using an epifluorescence microscope (SMZ18 with P2-SHR Plan Apo 1x, Nikon), illuminated with a solid-state light engine (SOLA SM2, Lumencor), and captured with a high-speed digital CMOS camera (Orca Flash 4.0 V2, Hamamatsu) controlled by NIS-Elements software (Nikon). A green fluorescent filter (P2-EFL GFP-B Filter Cube 470–535 nm, Nikon) was used. Images were acquired at 200 frames per second and digitally stored for offline analysis. For this study, we randomly selected up to 5 arterioles per cranial window experiment for further analysis, with baseline diameter ranging from 30 to 250 μm.

2.5. Ventilation

Ventilation was accomplished with a volume-controlled ventilator (Cato® Dräger, Lübeck, Germany) with the following settings: tidal volume (TV) of 10 ml/kg, PEEP 5, I/E ½, peak pressure 30cmH_2O and respiratory rate (RR) of 20–26/min adjusted to maintain an end-tidal carbon dioxide ($ETCO_2$) tension of 40 mmHg, verified by arterial blood gas sampling. We explored the $PaCO_2$ continuum to assess the effect of $PaCO_2$ on CVR. Hypocapnia was induced in steps of 5 mmHg by increasing the RR until the lowest obtainable value of $PaCO_2$. Hypercapnia was achieved in steps of 10 mmHg by slowly reducing the RR and increasing dead space ventilation by means of a swivel distal to the ventilation tubes, to arrive at the highest obtainable $PaCO_2$ value. Previous experiments have shown that hyperventilation is more delicate to achieve, therefore smaller steps of decreasing $PaCO_2$ are used (Van Hulst et al., 2002; Van Hulst et al., 2004). Each level of ETCO2 lasted for approximately 15 min and was checked by arterial blood gas when the level of $ETCO_2$ was stable for 10 min. We did not want a continuous increase or decrease of $ETCO_2$ since we wanted to give the animals time to recuperate and to establish a steady interstitial pH. In this short time span, we expect no interference of metabolic compensation. Two animals were first brought to hypercapnia and then slowly brought to hypocapnia. Three animals were first brought to hypocapnia and then brought to hypercapnia.

2.6. Statistical analysis

Analysis was performed using Excel (Microsoft, Washington, US) and

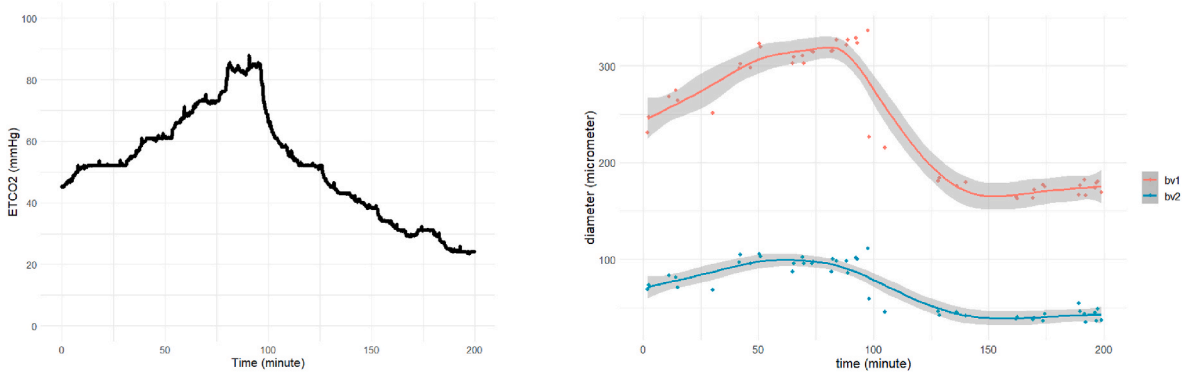

Fig. 1. The level of $ETCO_2$ is depicted over time for one animal, from normocapnia till hypercapnia (maximum 85 mmHg in this experiment), after which $ETCO_2$ is decreased to 25 mmHg. Both pial arterioles (bv1 and bv2, resp. 239 μm and 71 μm when $PaCO_2$ was 40 mmHg) dilate with increasing $ETCO_2$, however when RR is slightly increased there is a rapid decline of $ETCO_2$, and an even more rapid decline in diameter. By lowering $ETCO_2$, the diameter at $ETCO_2$ 60 mmHg is smaller compared to the starting point of $ETCO_2$ at 40 mmHg. This experiment took place over a period of 3 h, which prevented full metabolic compensation.

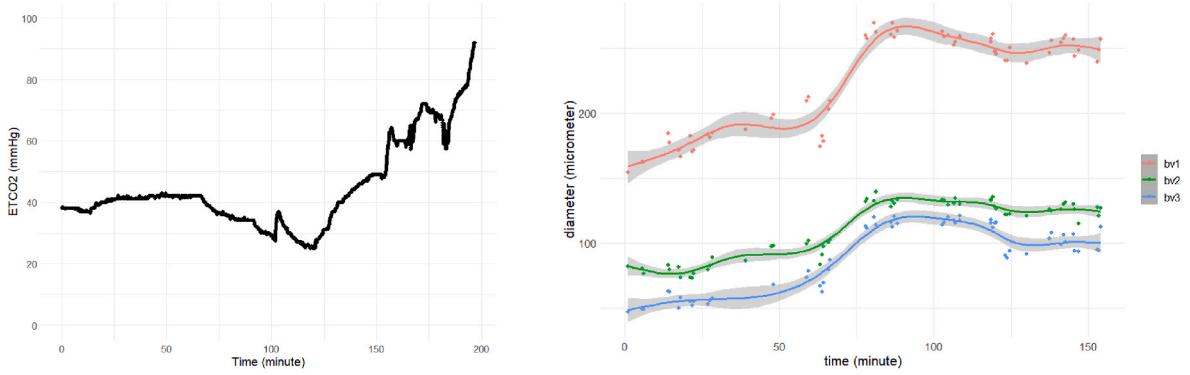

Fig. 2. The level of $ETCO_2$ is depicted over time for one animal, the three experiments in which hypocapnia was obtained first followed by hypercapnia look comparable. There is no change in diameter of the pial arterioles when decreasing $ETCO_2$ till 25 mmHg, independent on the baseline size of the blood vessel. When increasing $ETCO_2$ there is a rapid increase in diameter. Starting diameters at $PaCO_2$ of 40 mmHg in this experiment where 229 μm for bv1, 77 μm for bv2 and 152 μm for bv3.

R statistical software (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/). We used the following packages: tidyverse, lubridate, ggplot2, segmented, stringr, imputeTS, ggpubr.

2.7. Goal

We want to develop a solid piglet model to measure CA in a state of hyper- or hypocapnia. First, we want to confirm that this is possible solely by altering ventilation. Second, we want to know if $PaCO_2$ and $ETCO_2$ can be used as interchangeable parameters. Third, we want to investigate how $PaCO_2$ alters CVR of the arteriolar diameters, but also what the impact is on ICP, PbO_2 and LDF.

3. Results

We were able to bring $PaCO_2$ by hyperventilation to values as low as 25 mmHg (corresponding to a maximal RR of 51, though there are big differences between animals). Obtaining even lower $PaCO_2$ seemed impossible by further increasing RR, since the pigs became hemodynamically unstable with rapid changes in ABP along with tachycardia. They also started shivering, which led to a rise in $PaCO_2$ again. By hypoventilation combined with the use of a swivel, we were able to build up $PaCO_2$ as high as 90 mmHg (corresponding to a minimal RR of 14). 90 mmHg was the highest $PaCO_2$ measurable by our gas analyzer. When comparing the values of $ETCO_2$ with the values of $PaCO_2$, obtained by sampling arterial blood gases after 10 min of stabilization at the level of $ETCO_2$, no significant difference was found (two-tailed paired *t*-test with equal variance, $p = 0.93$).

In two out of five animals we first manipulated $PaCO_2$ to hyper- and then hypocapnia. The quality of the microscopic imaging in one of these animals turned out to be uninterpretable. In three out of five animals we manipulated first to hypo- and then to hypercapnia. Through the alteration of ventilation and $PaCO_2$, we did not see any significant changes in ABP (Pearson correlation $ETCO_2$ and ABP; $R = 0.19$). The direction of $PaCO_2$ change drove the change in pial arteriolar diameter, as displayed in Figs. 1 and 2. When using the Granger causality test, there was a significant causal relationship between $ETCO_2$ and both diameters in the hyper-then hypocapnia experiment, depicted in Fig. 1 ($p < 0.05$). There was no significant causal relationship in the other experiments between $ETCO_2$ and any of the measured diameters, all of which were hypo-then hyperventilation experiments.

Correlation coefficients between $PaCO_2$ on the one hand and PbO_2, ICP and LDF on the other hand were $R = 0.31$ (PbO_2), $R = 0.46$ (ICP) and $R = 0.58$ (LDF). In the higher ranges of $ETCO_2$, there seems to be an increase in PbO_2 and ICP. Since these experiments were limited in numbers as exploratory research, we cannot draw any statistical conclusion (Fig. 3).

4. Discussion

In the current study in which pial arteriolar diameters were studied through a cranial window in piglets while changing $PaCO_2$ by

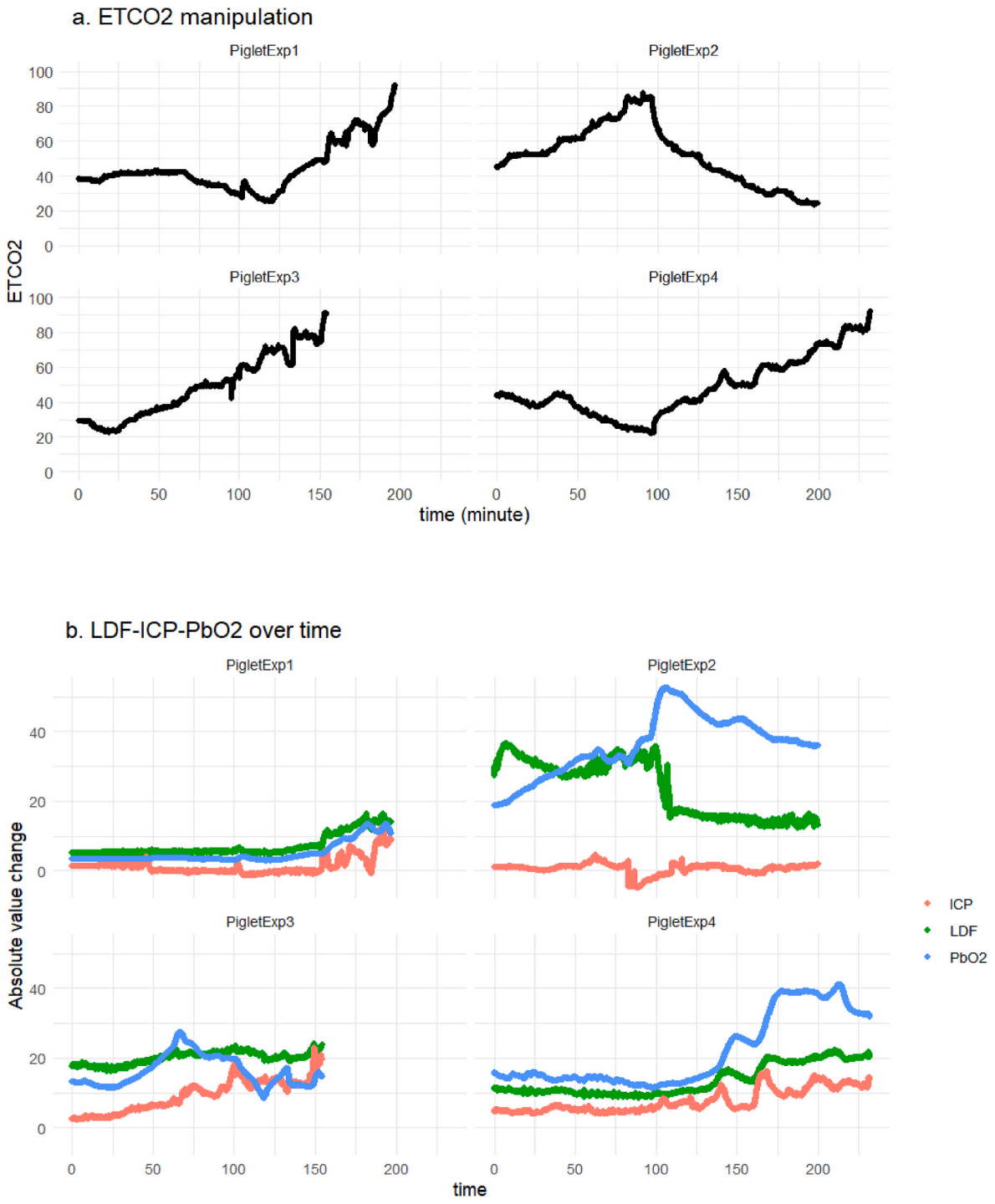

Fig. 3. 3a depicts $ETCO_2$ evolution over time in the four experiments. 3b depicts the absolute values of ICP, PbO_2 and LDF in the same time scale, however LDF is a relative number as it has no absolute numeric. We can see the relation of these values with $ETCO_2$, how they increase with hypercapnia and decrease with hypocapnia. Especially PbO_2 had very different absolute values at start ($PaCO_2$ of 40 mmHg), but there is a change seen in every experiment directly after adjusting $ETCO_2$.

ventilation settings, diameter changes followed the direction of $PaCO_2$ changes, but the effect of $PaCO_2$ on the diameters was not linear. It seems that rather the direction of change is the main driver of change in CBF. The biggest effects were seen in experiment 2, which is the only experiment where we first went to hypercapnia and then hypocapnia. This is the only experiment where a causality was proven between $ETCO_2$ and diameter by the Granger causality test, possibly since this is the only experiment where a clear decrease in diameter is proven. In the other experiments, we did not see a clear decrease in diameter when decreasing from 40 till 25 mmHg of $PaCO_2$, which is different than previous experiments have shown (Muizelaar et al., 1988; Brian, 1998; Kontos et al., 1977). The effect of different $PaCO_2$ levels on pial vasoreactivity in response to ABP changes (i.e. CA) has been explored in small animals, but is still totally unclear in humans and large animals, and the current experiments are a preparation for such investigation in our piglet cranial window model (Klein et al., 2019). Based on the current study, it was decided to investigate vasoreactivity to ABP changes at $PaCO_2$ levels of 25 mmHg and 60 mmHg. 25 mmHg was the

lowest achievable level of $PaCO_2$ with the animal remaining hemodynamically stable. 60 mmHg was chosen as the level of hypercapnia, since there was a clear arteriolar vasodilation at this level, while not being an extremely high level of $PaCO_2$ far outside the normal range.

The porcine cranial window model enables visualization of the pial arterioles, which constitute 21% of arterial resistance (Iadecola, 2017). The method however does not visualize the entire cerebrovascular tree. At present, no methods have been described that depict diameters of penetrating arterioles in real time with sufficient temporal and spatial resolution. Upstream, large arteries can be visualized with transcranial Doppler. Attempts were made to add transcranial Doppler measurements to the current set up, but with the instrumentation already used the piglet head did not allow for reliably securing the Doppler probe. The piglet head also proved too small for reliable application of Near Infrared Spectroscopy probes (NIRS). An estimation of cerebral blood flow was obtained by LDF. LDF is not often clinically used since it is very prone to displacement, but in a controlled laboratory setting it is easily applicable.

$PaCO_2$ is usually measured on arterial blood bases. In the present study, we found a strong and significant association between $ETCO_2$ and $PaCO_2$, and $ETCO_2$ is more practical to monitor in this complex setup. As stated, the actual driver of arteriolar diameter change is the interstitial pH, but its main determinant in the short term and in the present study is $PaCO_2$. Ideally, interstitial pH is included in the monitoring. Microdialysis is unpractical to add to the current model, since it takes too much time to collect readings from the pump that operates at a specific pace, and this is too slow to follow our adjustment of RR. Alternative pH probes were too bulky to apply on the small piglet brain. The Paratrend probe (initially produced by Diametrics Medical Ltd, UK), which can monitor brain interstitial pH and $PaCO_2$, is not available anymore. By modifying $PaCO_2$, indirectly we also modify PaO_2. On arterial blood gas, we made sure that the value of PaO_2 was always kept between 180 and 200 mmHg. As discussed in the introduction, due to the Bohr effect the decreasing pH increases the delivery of O_2 to tissues. Increasing pH shifts the curve to the left and withholds O_2 to separate from the Hb and leads to a local increase in CO_2 to counteract the increase in pH(Benner et al., 2022; Bö et al., 2020; Stocchetti et al., 2005; Patel et al., 2022)

Finally, we did not observe any change in ABP secondary to changing $PaCO_2$ and pH. This means that, in contrast to cerebral arterioles, the susceptibility of the systemic circulation to changes in $PaCO_2$ is minimal (Frö et al., 2018; Caldwell et al., 2021). This is important for our next experimental setup, where we want to investigate the effect of altering ABP to investigate CA in a state of hyper- or hypoventilation. If the level of $PaCO_2$ already had a clear influence on ABP it would blur our methods and results, since the influence of changing ABP is the main variable that we want to explore during the experiment. It will be of paramount importance to maintain a very stable level of $PaCO_2$ during the experiment, since small alterations could have an effect on CVR and may lead to a wrongful interpretation of our results.

5. Conclusion

Altering the RR to manipulate $PaCO_2$ by ventilation appeared to be feasible and reliable. We found that $ETCO_2$ reliably represents $PaCO_2$ to be used in our piglet cranial window model. Pial arteriolar diameter changes followed the direction of $PaCO_2$ changes, but the effect of $PaCO_2$ on the diameters was not linear.

Author contribution statement

SD, GM and BD designed the study concept. SD developed the protocol and performed all the experiments. VDS contributed to the experimental work and optimized the anesthesiology setup. SD analyzed the data and wrote the manuscript. GM and BD supervised the project.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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