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ORIGINAL ARTICLE

Mechanical and vasomotor properties of piglet isolated middle cerebral artery

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Dopamine concentration–response relations, mechanical characteristics, middle cerebral arteries, newborn, piglet, preterm, term, wire myography

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Introduction

Piglets are often used as experimental animal models to simulate the physiological response to hypotension in newborn infants. Previously, piglets have been used to describe cerebral autoregulation (Hahn et al. 2012, 2013) – a protective mechanism that ensures a fairly constant cerebral blood flow despite fluctuations in the blood pressure. Myogenic response is considered the most important component of this protective mechanism (Koller and Toth 2012). Unfortunately, knowledge regarding myogenic responses in middle cerebral arteries (MCA) from piglets is limited. To the best of our knowledge, MCA from newborn piglets have not been characterized by wire myography.

Abstract

Piglets are often used as experimental models for studying cerebrovascular responses in newborn infants. However, the mechanical characteristics of piglets' middle cerebral arteries (MCA) are not well characterized. Additionally, the vessels' response to dopamine, the most commonly used vasopressor in newborns, is not characterized in piglets' MCA. Finally, the influence of preterm birth on the dopamine response is not known. The aim of this current was to compare by wire myography the active and passive mechanical characteristics and dopamine concentration–response relations of MCAs isolated from preterm and term newborn piglets. Second-order branches of the MCA with a diameter <400 μm were chosen for study. The active and passive mechanical properties were comparable between vessels from six preterm (90% gestation, $n_{\text{segments}} = 11$) and nine term ($n_{\text{segments}} = 22$) newborn piglets. The response to increasing concentrations of dopamine was biphasic, starting with vasodilation in the 1 nmol/L–0.3 $\mu\text{mol/L}$ concentration range followed by vasoconstriction at higher concentrations. The response was very similar between the two groups. In conclusion, the mechanical properties of the MCA as well as the response to dopamine were comparable between term and 90% gestation preterm piglets.

Abbreviations

AUC, area under the curve; AWT, active wall tension; CRC, concentration–response curve; IC, internal circumference; I, internal diameter; MCA, middle cerebral arteries; PSS, physiological salt solution; PWT, passive wall tension..

Dopamine is the most commonly used vasopressor for treating hypotension and compromised blood flow in critically ill newborn infants. The aim of treating hypotension is to ensure a sufficient perfusion of the vital organs, especially the brain. In adult humans and cats, dopamine has been shown to induce constriction of cerebral arteries when the concentration is above 1 $\mu\text{mol/L}$ (Edvinsson et al. 1978; Toda 1983), thus it might seem contradictory to use dopamine when the purpose of treating hypotension is to raise the cerebral blood flow. However, dopamine's effect on MCA has never been established in a newborn model.

The responsiveness of cerebral arteries in preterm lambs and baboons differ from those of animals delivered at term (Hayashi et al. 1984; Docherty et al. 2001; Goyal

et al. 2012), therefore similar differences may be present in term versus preterm piglet arteries.

The aim of this study was to describe mechanical properties and response to dopamine in MCA from term and preterm newborn piglets using wire myography. Our hypothesis was that MCA from term and preterm piglets differed due to structural and functional immaturity of the preterm piglets' arteries.

Briefly, we found that active and passive mechanical characteristics of MCA from preterm and term newborn piglets were comparable. Increasing dopamine concentration caused a biphasic response starting with vasodilation at low concentrations, followed by vasoconstriction at higher concentrations. These curves were also comparable between the two age groups.

Materials and Methods

Solutions and chemicals

Physiological salt solution (PSS) had the following composition (in mmol/L): NaCl 119, NaHCO₃ 25, KCl 4.7, CaCl₂ 1.5, KH₂PO₄ 1.18, MgSO₄·7H₂O 1.17, ethylenediaminetetraacetic acid (EDTA) 0.027, and glucose 5.5, with pH adjusted to 7.4. Ca²⁺-free PSS was similar to PSS except that CaCl₂ was replaced by 0.01 mmol/L ethyleneglycol-bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). K-PSS was prepared by replacing all sodium in PSS with an equimolar amount of potassium resulting in a total extracellular K⁺ concentration of 125 mmol/L.

Dopamine hydrochloride 40 mg/mL (for intravenous injection) was obtained from Orion Corporation (Espoo, Finland). Noradrenaline tartrate (for slow intravenous infusion) and bradykinin were obtained from Sigma-Aldrich (St Louis, MO). Bradykinin was dissolved in distilled H₂O and aliquots were stored frozen at -20°C. Dilutions were prepared just before the experiments.

Animals

All animal procedures were carried out in accordance to the national law and guidelines. A total of nine preterm and 15 term piglets were used in this study. Term piglets, below 48 h old, were randomly taken from the sow at the experimental days. Preterm piglets were delivered at approximately 90% of full gestational age (full gestation 116 days), and examined when they were 4–5 days old, after participation in another study that focused on how the newborn gastrointestinal tract responds to different nutritional interventions (Sangild *et al.* 2013). Killing was carried out by anesthesia with Zoletil mix (1 mg kg⁻¹, Virbac, Carros, France) combined with an overdose of

pentobarbitale (200 mg mL⁻¹, Glostrup apotek, Denmark).

Ethical considerations

Newborn piglets are often used as an experimental model for newborn infants and in that case, we have to consider if gestational age matters. Premature newborn infants are more vulnerable and have a higher risk of intracranial bleeding (Bassan 2009). Therefore, it seems reasonable to consider that the arteries may differ. When it comes to term piglets, one piglet can be examined independently, whereas examination of preterm piglets requires caesarian section and delivery of a whole litter (~10–15 piglets) and, in some experimental protocols, only one or two piglets can be examined per day. Therefore, to minimize animal number, we compared MCA from term piglets with preterm piglets first used for a study of gastrointestinal function.

Tissue preparation

The brain was gently removed from the skull and immersed in pre-oxygenated ice-cold (4°C) PSS. MCA was identified and second order MCA branches were isolated and immersed in pre-oxygenated ice-cold Ca²⁺-free PSS. One investigator (VRE) isolated all segments.

Wire myography

MCA segments (1–2 mm long) were mounted on two stainless steel wires (diameter: 25 μm) in an organ bath of a small vessel wire myograph (Danish Myo Technology A/S, Aarhus, Denmark). The wires were connected to a force transducer and micrometer. In the organ bath, the isolated MCA segments were allowed to equilibrate in 37°C oxygenated (5% CO₂/95% O₂) PSS for at least 30 min.

IC–tension relationship studies

Relationship between internal circumference of the artery (IC) and passive and active tension was conducted according to the previous study by Mulvany and Halpern (1977) and as recently described by our research group (Outzen *et al.* 2015). The optimal IC (IC₀) with the highest active wall tension development (ΔAWT) was estimated. ΔAWT is the difference between the active and passive wall tension in Nm⁻¹. Passive wall tension (PWT) was determined in Ca²⁺-free PSS during stepwise increase in IC by the micrometer (example trace shown in Fig. 1). For each segment, the ratio IC₀/IC₁₀₀ was determined with the aim to establish the optimal IC for maximal

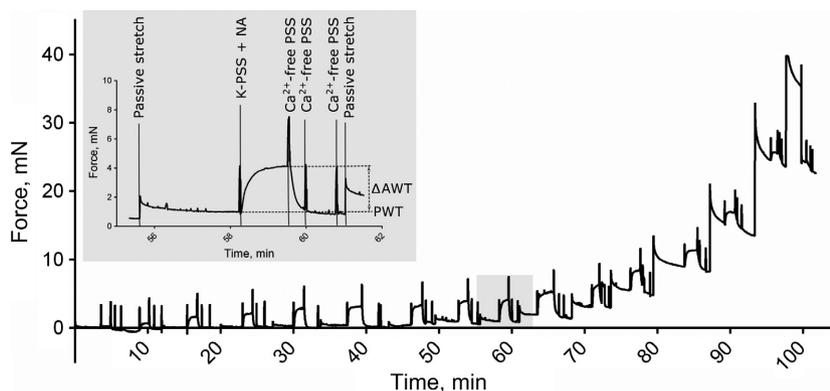


Figure 1. Representative trace of length-tension study. PWT was determined in Ca^{2+} -free PSS during stepwise increase in IC by the micrometer. At each step, the segments were maximally contracted with a depolarizing bicarbonate buffer solution (K-PSS) containing $10 \mu\text{mol/L}$ noradrenaline (NA) and ΔAWT was estimated. The gray box illustrates one sequence (also marked with a gray box on the trace) for determination of PWT and ΔAWT . AWT, active wall tension; PWT, passive wall tension.

ΔAWT . IC_{100} is the IC the artery would have had if it was exposed to a passive transmural pressure of 100 mmHg.

Results from the passive IC–tension study was fitted to an exponential growth equation: $\text{PWT} = \text{PWT}_0 \times e^{[k \cdot \text{IC}]}$, where PWT_0 is PTW at IC_0 , and k , elastic modulus, is a constant that related to the slope of the IC–passive tension relation reflecting the stress produced in response to an applied strain (Mulvany and Aalkjær 1990). Results from the IC–active tension study was fitted to a Gaussian distribution equation: $\Delta\text{AWT} = \text{Amplitude} \times \exp(-0.5 \cdot ((\text{IC} - \text{mean})/\text{SD}))^2$, where amplitude is the height of the center of the curve, mean is the IC value at the center of the curve (IC_0), and SD is the width of the distribution.

Results are presented as internal diameter and are defined as IC divided by π (Mulvany and Halpern 1977).

Dopamine concentration–response curve

Normalization was performed as described by Mulvany and Halpern (1977), with the slight modification of only stretching the MCA to the maximum of 9 kPa in order to avoid overstretching and thereby compromising the functionality of the MCA.

The protocol was initiated by stimulating the arteries with K-PSS three times. The pharmacodynamic characteristic to cumulative concentration of dopamine (1 nmol/L – 0.3 mmol/L) was examined (half-log increments), and the response to increasing concentrations of dopamine was described as a percentage of maximal response to K-PSS. The dopamine concentration–response curve (CRC) was biphasic with vasodilation at low concentration and vasoconstriction at relatively higher concentrations. The vasoconstrictive response to cumulative concentrations of

dopamine was fitted to the four-parameter sigmoid equation: $E/E_{\text{max}} = A[M]^{n_H} / (A[M]^{n_H} + \text{EC}_{50}[M]^{n_H})$, where E_{max} is the maximal response developed to the agonist, in this case dopamine; $A[M]$ is the concentration of dopamine, and n_H , Hills coefficient, is a curve-fitting parameter (Kenakin 1997). Sensitivity to dopamine is expressed as pEC_{50} value, where $\text{pEC}_{50} = -\log(\text{EC}_{50} [\text{M}])$, and $\text{EC}_{50} [\text{M}]$ is the molar concentration of agonist required to produce half-maximal response (Kenakin 1997). The spontaneous myogenic tone in each MCA segment was calculated as the difference in the resting tone of MCAs in PSS buffer and Ca^{2+} -free buffer.

Assessment of endothelial function

At the end of the dopamine CRC experiment, the endothelial response was assessed by bradykinin (0.1 – $10 \mu\text{mol/L}$) induced vasodilation (Görlach and Wahl 1996). Bradykinin has previously been used to test endothelial function in MCA from piglets (Martínez-Orgado et al. 1998). Both bradykinin and substance P induce endothelium-dependent vasodilation in porcine cerebral arteries (Pacicca et al. 1992), whereas acetylcholine does not induce vasodilation of porcine cerebral arteries with intact endothelium (Gräser et al. 1986). We considered the artery to have optimal endothelial function if bradykinin induced $>20\%$ relaxation compared to the resting tension in Ca^{2+} -free PSS.

Data and statistical analysis

Results are given as mean \pm SEM and n is the number of segments, with N , the number of animals, also provided. Comparison between two groups was done by two-tailed student's t -test. Statistical significance was considered if

Table 1. Characteristics of second-order middle cerebral arteries from term piglets with and without functional endothelium.

	Insufficient endothelial function	Endothelial function	<i>P</i> -value
<i>n</i> (<i>N</i>)	8 (4)	14 (7)	
Endothelial response (%)	5 ± 3	56 ± 8	<0.001
<i>l</i> ₀ (μm)	334 ± 20	294 ± 15	0.121
<i>l</i> ₁₀₀ (μm)	453 ± 29	350 ± 26	0.022
PWT ₀ (N·m ⁻¹)	1.0 ± 0.2	1.6 ± 0.3	0.169
ΔAWT ₀ (N·m ⁻¹)	1.3 ± 0.1	1.5 ± 0.3	0.725
Elastic modulus (N·m ⁻¹ ·μm ⁻¹)	0.0115 ± 0.0008	0.0118 ± 0.0009	0.826
IC ₀ /IC ₁₀₀	0.75 ± 0.05	0.91 ± 0.09	0.246

Results are given as mean ± SEM. *n* is the number of segments analyzed and *N* is the number of piglets. Comparison between the two groups was performed with *t*-test. Endothelial response is given as % relaxation compared to the tension in Ca²⁺-free PSS. *l*₀ represents the internal diameter (*l*) where the arteries elicit their maximal active wall tension (ΔAWT₀). PWT₀ is the passive wall tension at *l*₀. *l*₁₀₀ is an extrapolated value that describes the internal diameter that the artery would have had if the artery had been exposed to a passive transmural pressure of 100 mmHg. IC₀/IC₁₀₀ is the optimal normalization ratio.

the *P*-value was below 0.05. GraphPad 6.07 and SPSS 22 were used for the calculations and statistical analyses.

Compliance with design and statistical analysis requirements

Statistical analysis (*t*-test) was only performed on groups of *n* > 5. Only arteries with an internal diameter <400 μm and where stimulation with K-PSS elicited a minimum intraluminal pressure (P_{KPSS}) > 1 Nm⁻² were included. We examined either term or preterm piglets at the experimental days. Chose of organ baths in the wire myography experiments was randomized. The response to cumulative additions of dopamine in the wire myograph

was normalized to the maximal response elicited by K-PSS in each vessel segment.

Results

IC-tension relationships

The relationship between IC and tension was examined in MCA from preterm and term newborn piglets (Fig. 1). Fourteen out of 22 MCA segments from term piglets had functional endothelium as assessed by responsiveness to bradykinin. In this age group, passive and active characteristics were comparable for MCA with and without functional endothelium (Table 1, Fig. 2), hence the results from segments with and without functional endothelium were pooled for subsequent comparisons of active and passive characteristics of MCA from term and preterm piglets.

The passive tension of term and preterm MCA at IC₀ (PWT₀) were similar (Fig. 3A and Table 2), but the term piglets had a significantly higher elastic modulus compared to the preterm piglets (*P* = 0.008).

The relative active wall tension relationships to relative internal diameter (Fig. 3B) were shown to be bell-shaped. The curves from the preterm and term newborn piglets were identical and both curves reach comparable maximal active wall tension (ΔAWT₀) and IC₀ (Table 2).

Dopamine CRC

Dopamine at the concentrations range of 1 nmol/L–0.3 μmol/L evoked a vasodilatory response in wire-myograph mounted MCA, whereas higher concentrations induced vasoconstriction (Fig. 4). Dopamine concentration-response curves were compared for MCAs from term piglets with and without functional endothelium. Arteries with insufficient endothelial function displayed a lower spontaneous tone in Ca²⁺-free PSS compared to the group with functional endothelium, otherwise the groups were

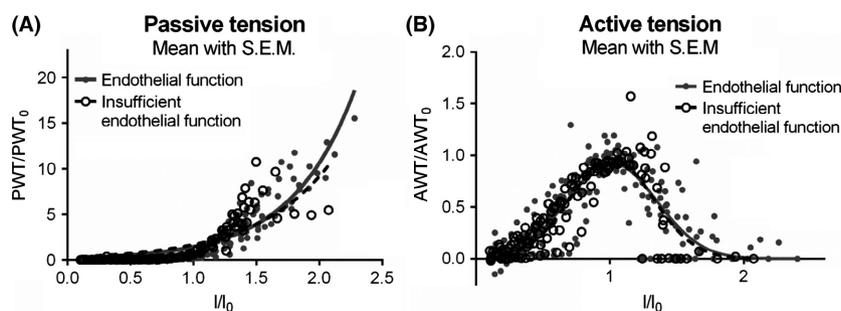


Figure 2. Relative passive and active internal circumference–wall tension relationship for middle cerebral arteries from term piglets with and without functional endothelium. (A) Relation between relative (PWT/PWT₀) and relative diameter (*l*/*l*₀). (B) Relation between relative AWT/AWT₀ and *l*/*l*₀. AWT, active wall tension; PWT, passive wall tension; AWT, active wall tension.

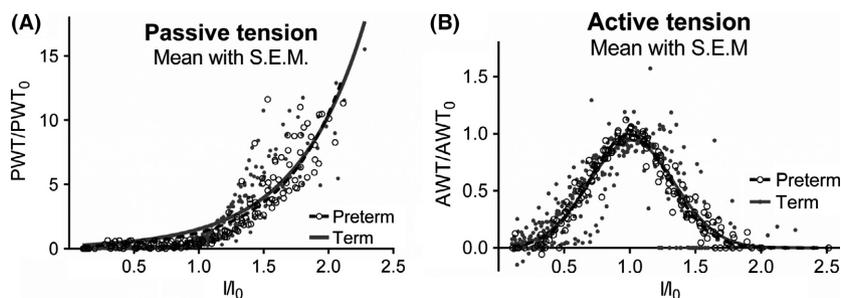


Figure 3. Relative passive and active internal circumference–wall tension relationship. Comparison between middle cerebral arteries from term and preterm piglets. (A) Relation between relative PWT/PWT₀ and relative diameter (I/I_0). (B) Relation between relative AWT/AWT₀ and I/I_0 . Solid dots and line represent the measurements from term piglets, and circles and dotted line represent preterm piglets. AWT, active wall tension; PWT, passive wall tension.

Table 2. Characteristics of second-order middle cerebral arteries from term and preterm newborn piglets.

	Preterm	Term	<i>P</i> -value
<i>n</i> (<i>N</i>)	11 (6)	22 (9)	
Endothelial response (% (<i>n</i>))	68 ± 12 (9)	56 ± 8 (14)	
l_0 (μm)	309 ± 14	308 ± 12	0.953
l_{100} (μm)	367 ± 22	387 ± 22	0.572
PWT ₀ (N·m ⁻¹)	1.4 ± 0.2	1.4 ± 0.2	0.938
ΔAWT ₀ (N·m ⁻¹)	1.2 ± 0.2	1.4 ± 0.2	0.391
Elastic modulus (N·m ⁻¹ ·μm ⁻¹)	0.0089 ± 0.0007	0.0117 ± 0.0006	0.008
IC ₀ /IC ₁₀₀	0.86 ± 0.04	0.86 ± 0.06	0.988

Results are given as mean ± SEM. *n* is the number of segments analyzed and *N* is the number of piglets. Comparison between the two groups was performed with *t*-test. Endothelial response is given as *n* with functional endothelial response and the % relaxation compared to the tension in Ca²⁺-free PSS. l_0 represents the internal diameter (*l*) where the arteries elicit their maximal active wall tension (ΔAWT₀). PWT₀ is the passive wall tension at l_0 . l_{100} is an extrapolated value that describes the *l* that the artery would have had if the artery had been exposed to a passive transmural pressure of 100 mmHg. IC₀/IC₁₀₀ is the optimal normalization ratio.

comparable. Therefore, the data from arteries with and without functional endothelium were pooled when comparing term and preterm piglets.

The response to cumulative concentrations of dopamine was very similar in MCA from term and preterm piglets (Fig. 6). There was no difference in the vasoconstrictive response to dopamine with regard to mean pEC₅₀ value, Hill slope, and E_{max} when comparing results from preterm and term newborn piglets (Table 4).

Discussion and Conclusion

In this study, we have shown that MCA from preterm and term newborn piglets elicited comparable passive and active characteristics as well as a comparable response to dopamine.

Dopamine acts by stimulating both dopaminergic receptors as well as α - and β_1/β_2 -adrenoceptors (Olsen 1998; Overgaard and Dzavik 2008), and it has previously been shown that the predominant effects of dopamine are dose-related. In adults, low infusion rate of dopamine (0.5–3 μg/kg/min) leads to vasodilation caused by stimulation of dopaminergic D₁ postsynaptic receptors in the renal, mesenteric, coronary, and cerebral vascular beds (Olsen 1998; Overgaard and Dzavik 2008). Higher infusion rates stimulate β -adrenoceptors, resulting in peripheral vasodilation; however, in isolated cerebral arteries, blocking the β -receptors with propranolol did not affect the vasodilation induced by dopamine (Edvinsson et al. 1978; Toda 1983), indicating that dopamine-induced vasodilation is primarily caused by dopamine receptors. At infusion rates above 10 μg/kg/min, α -adrenoceptors are stimulated resulting in vasoconstriction and rise in mean arterial blood pressure (Overgaard and Dzavik 2008). Even though dopamine has been observed to have similar effect in newborns (Seri 1995), it has also been demonstrated that in the preterm newborn infants, dopamine at low doses has a pronounced effect on α - and dopamine-receptors, with minimal activity at β -receptors (Seri et al. 1984; Seri 1995). Therefore, concentration-related responses observed in adults cannot be extrapolated into an assumed response in newborns.

IC–tension relationship

The only parameter that was significantly different between MCA from preterm and term newborn piglets in the IC–tension relationship study was that MCA from term piglets had significantly higher elastic modulus compared to MCA from preterm piglets (Table 2, $P = 0.008$). However, the statistical difference between these two groups is caused by low measurement variation, and may not actually impact on the passive characteristics of the arteries.

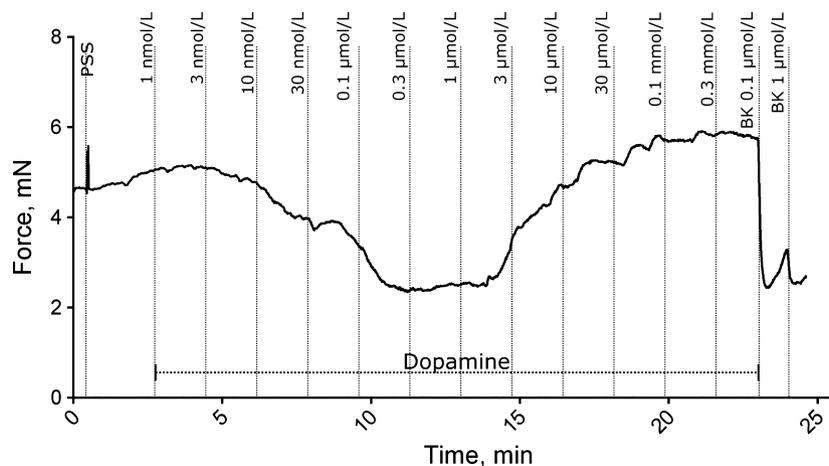


Figure 4. Representative trace of dopamine concentration-responses of middle cerebral artery from term piglet. The artery segment was exposed to cumulative concentration of dopamine (1 nmol/L–0.3 mmol/L). This segment had a biphasic response starting with vasodilation in the 3 nmol/L–0.1 μ mol/L concentration range followed by vasoconstriction at higher concentrations.

Table 3. Dopamine concentration-response for term piglets with and without functional endothelium.

	Insufficient endothelial function	Endothelial function	P-value
n (N)	8 (4)	14 (7)	
Resting tension in Ca^{2+} -free PSS ($N\cdot\text{m}^{-1}$)	0.21 ± 0.05	0.43 ± 0.07	0.047
Resting tension in PSS ($N\cdot\text{m}^{-1}$)	0.88 ± 0.14	0.74 ± 0.12	0.467
Spontaneous myogenic tone ($N\cdot\text{m}^{-1}$)	0.67 ± 0.13	0.49 ± 0.14	0.414
ΔT_{KPSS} ($N\cdot\text{m}^{-1}$)	1.01 ± 0.15	0.78 ± 0.21	0.376
pEC_{50} (dopamine)	4.93 ± 0.20	5.48 ± 0.42	0.356
Hill slope	0.85 ± 0.08	1.43 ± 0.46	0.360
E_{max} (%)	52 ± 11	51 ± 11	0.970

ΔT_{KPSS} is the difference between the maximal tension elicited by K-PSS and the resting tension in Ca^{2+} -free PSS. E_{max} for dopamine (%) is calculated as relative response to maximum steady-state contraction induced by KPSS.

The optimal normalization ratio, $\text{IC}_0/\text{IC}_{100}$ ratio, was comparable in the two groups (Table 2), and our results are in line with the well-described optimal normalization ratio for rat and mouse small mesenteric resistance arteries (Mulvany and Halpern 1977; Outzen et al. 2015). Even though it has been reported that the contractile response to K-PSS was unaffected, regardless of the normalization setting chosen between 0.7 and 0.9 in fetal ovine MCA (Docherty et al. 2001), we cannot rule out the difference in normalization setting between vessels isolated from different species or vessels of different origin (Slezák et al. 2010).

We did not detect any differences between arteries with and without functional endothelium.

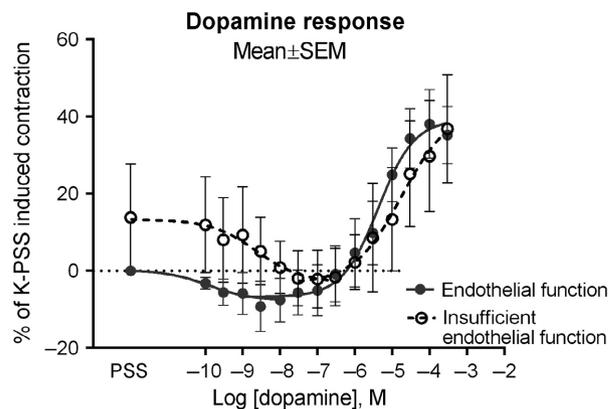


Figure 5. Cumulative concentration-response to dopamine. Comparison between term middle cerebral artery with and without functional endothelium. Arteries with insufficient endothelial function (circles and dotted line) had an insignificant higher tension in PSS and a more pronounced vasodilation caused by dopamine compared to the group with functional endothelium (solid dots and line). Otherwise the groups were similar.

Dopamine CRC

When comparing MCA with and without sufficient endothelial function, MCAs without functional endothelium elicited a lower tone in Ca^{2+} -free PSS and had an insignificant higher spontaneous myogenic tone in PSS (Table 3, Fig. 5). As a consequence, the observed vasodilation caused by dopamine was more pronounced in this latter group (no functional endothelium), however, this difference was not significant.

MCA from both preterm and term newborn piglets elicited spontaneous myogenic tone in PSS, which was dependent on extracellular Ca^{2+} as MCA relaxed upon

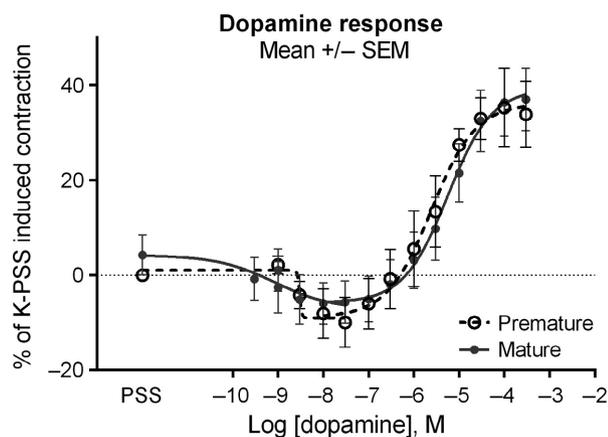


Figure 6. Cumulative concentration–response to dopamine. Comparison between middle cerebral arteries from term and preterm piglets. Response to cumulative concentration of dopamine (1 nmol/L–0.3 mmol/L) was similar between the term piglets (solid dots and line) and preterm piglets (circles and dotted line).

Table 4. Dopamine concentration–response curve.

	Preterm	Term	<i>P</i> -value
<i>n</i> (<i>N</i>)	11 (6)	22 (9)	
Resting tension in Ca ²⁺ -free PSS (<i>N</i> ·m ⁻¹)	0.56 ± 0.12	0.35 ± 0.05	0.074
Resting tension in PSS (<i>N</i> ·m ⁻¹)	1.42 ± 0.19	0.90 ± 0.10	0.010
Spontaneous myogenic tone (<i>N</i> ·m ⁻¹)	0.86 ± 0.19	0.56 ± 0.10	0.125
Δ <i>T</i> _{KPSS} (<i>N</i> ·m ⁻¹)	1.22 ± 0.14	0.86 ± 0.15	0.128
pEC ₅₀ (dopamine)	6.01 ± 0.16	5.42 ± 0.25	0.120
Hill slope	1.88 ± 0.42	1.40 ± 0.33	0.393
<i>E</i> _{max} (%)	51 ± 10	52 ± 8	0.959

Δ*T*_{KPSS} is the difference between the maximal tension elicited by K⁺PSS and the resting tension in Ca²⁺-free PSS. *E*_{max} for dopamine (%) is calculated as relative response to maximum steady-state contraction induced by KPSS.

exposure to Ca²⁺-free PSS (Sheykhzade et al. 2012) (Table 4). This is in contrast to cerebral arteries from adult humans and cats that needed a precontraction tone to demonstrate dopamine-induced vasodilation (Edvinsson et al. 1978; Toda 1983). We did not precontract the arteries and found a vasodilatory effect of dopamine at the concentration range 1 nmol/L–0.3 μmol/L (Fig. 2). In the studies performed in cats, EC₅₀ for dopamine was 0.85 μmol/L (Edvinsson et al. 1978), and in human cerebral arteries, dopamine-induced vasodilation occurred at concentrations up to 10 μmol/L (Toda 1983). However, actively inducing precontraction of the arteries might affect the subsequent vasodilatory response, and such experimental differences could possibly explain the observed differences between these studies and the data presented herein.

At higher concentrations of dopamine, we observed a contractile response that was again comparable in the preterm and term piglets' MCA (Fig. 6) with pEC₅₀ (dopamine)-values of 6.01 ± 0.16 and 5.42 ± 0.25, respectively (Table 4). In cats, pEC₅₀ (dopamine) was 4.4 (Edvinsson et al. 1978) and in human cerebral arteries dopamine induced a slight contraction at concentrations above 10 μmol/L (Toda 1983). This difference indicates that the cerebral arteries from our newborn piglets were more sensitive to dopamine-induced contraction compared to the adult models. In agreement with this observation, MCA from newborn baboons, born at term as well as prematurely, have a higher sensitivity to contractile substances compared to adult baboons (Hayashi et al. 1984).

Even though evidence exist that the myogenic response changes with gestational and postnatal age (Hayashi et al. 1984; Docherty et al. 2001; Goyal et al. 2012), we were not able to detect a difference in the IC–tension relationship or the dopamine concentration–response curves between term and preterm piglets. This may reflect that difference in age was too small in our two groups. The preterm piglets were delivered at 90% of full gestational age and were 4–5 days old, whereas the term piglets were below 48 h. A total of 90% of full gestational age is not a very good preterm model. However, examination of cerebral arteries from preterm piglets raises some ethical issues, as described in Materials and Methods, and therefore, we decided to use cerebral arteries from preterm piglets that had been enrolled in another study. In that study, the piglets were required to survive for some days, and had the piglets been delivered at a younger age, their viability would have decreased (Sangild et al. 2013). Having said that, it would be valuable to examine the preterm piglets at younger gestational and postnatal ages.

In conclusion, we found that active and passive mechanical characteristics of MCA from preterm and term newborn piglets were comparable. The optimal normalization ratio/setting was established and was comparable to the well-described ratios calculated in rat mesenteric resistance arteries. Increasing concentrations of dopamine caused a biphasic response, starting with vasodilation at low concentrations followed by vasoconstrictions at relatively higher concentrations. Furthermore, the curves were comparable between the two age groups.

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Disclosure

None to declare.

References

- Bassan H (2009). Intracranial hemorrhage in the preterm infant: understanding it preventing it. *Clin Perinatol Elsevier Ltd*;36: 737–762.
- Docherty CC, Kalmar-nagy J, Engelen M, Nathanielsz PW, Cheryl C, En- M (2001). Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep. *Am J Physiol Regul Integr Comp Physiol* 280: R554–R562.
- Edvinsson L, Hardebo J, McCulloch J, Owman C (1978). Effects of dopaminergic agonists and antagonists on isolated cerebral blood vessels. *Acta Physiol Scand Scand* 104: 349–359.
- Görlach C, Wahl M (1996). Bradykinin dilates rat middle cerebral artery and its large branches via endothelial β_2 receptors and release of nitric oxide. *Peptides* 17: 1373–1378.
- Goyal R, Henderson DA, Chu N, Longo LD (2012). Ovine middle cerebral artery characterization and quantification of ultrastructure and other features: changes with development. *Am J Physiol Regul Integr Comp Physiol* 15: R433–R445.
- Gräser L, Leisner H, Tiedt N (1986). Absence of role of endothelium in the response of isolated porcine coronary arteries to acetylcholine. *Cardiovasc Res* 20: 299–302.
- Hahn GH, Heiring C, Pryds O, Greisen G (2012). Cerebral vascular effects of hypovolemia and dopamine infusions: a study in newborn piglets. *Acta Paediatr* 101: 736–742.
- Hahn GH, Hyttel-Sorensen S, Petersen SM, Pryds O, Greisen G (2013). Cerebral effects of commonly used vasopressor-inotropes: a study in newborn piglets. *PLoS ONE* 8: e63069.
- Hayashi S, Park MK, Kuehl TJ (1984). Higher sensitivity of cerebral arteries isolated from premature and newborn baboons to adrenergic stimulation. *Life Sci* 35: 253–260.
- Kenakin T (1997). *Pharmacological analysis of drug-receptor interaction*, 3rd ed.. Lippincott-Raven, Philadelphia.
- Koller A, Toth P (2012). Contribution of flow-dependent vasomotor mechanisms to the autoregulation of cerebral blood flow. *J Vasc Res* 49: 375–389.
- Martínez-Orgado J, González R, Alonso MJ, Rodríguez-Martínez MA, Sánchez-Ferrer CF, Marín J (1998). Endothelial factors and autoregulation during pressure changes in isolated newborn piglet cerebral arteries. *Pediatr Res* 44: 161–167.
- Mulvany MJ, Aalkjær C (1990). Structure and function of small arteries. *Am J Physiol* 70: 921–961.
- Mulvany MJ, Halpern W (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ Res* 41: 19–26.
- Olsen NV (1998). Effect of dopamine on renal haemodynamic tubular function and sodium excretion in normal humans. *Dan Med Bull* 45: 282–297.
- Outzen EM, Zaki M, Abdolazizadeh B, Sams A, Boonen HCM, Sheykhzade M (2015). Translational value of mechanical and vasomotor properties of mouse isolated mesenteric resistance-sized arteries. *Pharmacol Res Perspect* 3: e00200.
- Overgaard CB, Dzavík V (2008). Inotropes and vasopressors: Review of physiology and clinical use in cardiovascular disease. *Circulation* 118(10): 1047–1056.
- Pacicca C, von der Weid PY, Beny JL (1992). Effect of nitro-L-arginine on endothelium-dependent hyperpolarizations and relaxations of pig coronary arteries. *J Physiol* 457: 247–256.
- Sangild PT, Thymann T, Schmidt M, Stoll B, Burrin DG, Buddington RK (2013). The preterm pig as a model in pediatric gastroenterology. *J Anim Sci* 91: 4713–6359.
- Seri I (1995). Cardiovascular, renal, and endocrine actions of dopamine in neonates and children. *J Pediatr* 126: 333–344.
- Seri I, Tulassay T, Kizsel J, Machay T, Csömör S (1984). Cardiovascular response to dopamine in hypotensive preterm neonates with severe hyaline membrane disease. *Eur J Pediatr* 142: 3–9.
- Sheykhzade M, Simonsen AH, Boonen HCM, Outzen EM, Nyborg NCB (2012). Effect of ageing on the passive and active tension and pharmacodynamic characteristics of rat coronary arteries: age-dependent increase in sensitivity to 5-HT and K. *Pharmacology* 90(3–4): 160–168.
- Slezák P, Waczulíková I, Balis P, Púzserová A (2010). Accurate normalization factor for wire myography of rat femoral artery. *Physiol Res* 59: 1033–1036.
- Toda N (1983). Dopamine vasodilates human cerebral artery. *Experientia* 39: 1131–1132.