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CLINICAL STUDY

Chronic hypoxia increases fetoplacental vascular resistance in rat placenta perfused with blood

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ABSTRACT
BACKGROUND: Chronic hypoxia elevates vascular resistance on the fetal side of the placenta. However, when a low-viscosity perfusate is used, the increase in resistance caused by chronic hypoxia is relatively small (12 mmHg). In the present study, we tested the hypothesis that perfusion with more viscous fluid (blood) will reveal more substantial effect of chronic hypoxia.
METHODS: Using an isolated, dually perfused rat placenta perfused at a constant flow rate with homologous blood, perfusion pressure on the fetal side was measured. Then, the relationship between perfusion pressure and flow (P/Q) was determined.
RESULTS: Fetoplacental vascular resistance was increased by chronic hypoxia (10 % O_2) during the last third of gestation. This was observed when the placentas were perfused with a low viscosity Krebs solution and more enhanced when perfused with blood. Nevertheless, we found no clear advantage for using blood instead of Krebs solution to study the effects of hypoxia on the fetoplacental vasculature. The elevation of fetoplacental vascular resistance caused by chronic hypoxia was at least partly resistant to acute reoxygenation.
CONCLUSION: Using blood for the perfusion of the isolated rat placenta does not confer any clear methodological advantage over using Krebs solution *(Tab. 2, Fig. 2, Ref. 21)*. Text in PDF *www.elis.sk.*
KEY WORDS: placenta, blood perfusion, chronic hypoxia, pressure/flow relationship, intrauterine growth restriction.

Introduction

One of the main persisting problems of neonatology are intrauterine growth restriction (IUGR) and preeclampsia (1, 2). There is a solid evidence that lower than normal birth weight is associated with a number of neonatal and even adult morbidities (3, 4). In utero hypoxia – arising from persistent maternal hypoxemia or from chronic maternal vascular disorders (1, 5) – is commonly perceived as an important causative factor leading to fetal growth restriction (6). It is assumed that intrauterine hypoxia results in vasoconstriction of the vessels of the fetal side of the placenta (7) (similar to the hypoxic vasoconstriction in the lungs) and consequent placental hypoperfusion and fetal undernutrition. However, there is little direct evidence for this mechanism. Recently, we showed that exposure of pregnant rats to chronic hypoxia caused an increased fetoplacental vascular resistance in a low-viscosity fluid-perfused rat placenta (8). However, in that study, the difference between the chronically hypoxic and normoxic groups, albeit significant, was not numerically very large (~ 12 mmHg). It is possible that the low viscosity of the perfusate (as one of the contributors to resistance) might have contributed to this relatively small magnitude of the change induced by chronic hypoxia.

To better approximate the in vivo conditions, we therefore developed a model of a rat placenta perfused with blood. We then used it to measure the resistance of fetoplacental vessels in pregnant rats kept in normoxia and chronic hypoxia.

Materials and methods

All experiments were performed using pregnant female rats of the Wistar strain (Biotest, Konarovice, Czech Republic). All animal handling and study procedures were approved by the ethical committee of the 2nd Faculty of Medicine, Charles University in Prague.

The animals were located at our laboratory for acclimatization for 1 to 2 weeks before the expected date of delivery. The rats were divided into the two groups. The normoxic control group (n = 6) spent the whole gravidity in the atmospheric air, while the hypoxic group (n = 8) spent the last 7 days of pregnancy (term = 21 days) in the hypoxic normbaric chamber (F_iO_2 = 0.1) (9). One day before delivery (day 20 of gravidity) each rat was anesthetized with Thiopental (50 mg/kg i.p.). The model of isolated, dually perfused placenta (10) was prepared as previously described (8, 11). The rat was placed in a bath of Ringer's solution kept at 37 ºC during the

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entire experiment. After opening the abdominal wall by midline incision, one horn of the uterus was exposed and padded by folded piece of mull. A suitable placenta with its fetus was selected by visually inspecting the exposed uterus. Its afferent uterine artery was cannulated with a polyethylene 24-gauge catheter and perfused with Krebs solution saturated with normoxic gas mixture (21 % O_2, 5 % CO_2 and 74 % N_2). The flow rate was gradually increased to 1 ml/min and then kept constant. The other vessels separating the chosen placenta-fetus unit were ligated and the uterine vein was punctured to allow free outflow of the perfusate. Then the uterus was opened and the fetus was exposed. The umbilical cord was separated from the omphalomesenteric vessels and both the umbilical artery and vein were cannulated with a 24-gauge polyethylene catheter. The preparation was perfused via the umbilical artery at 1 ml/min with the same perfusate as the maternal side of the placenta from the same reservoir equilibrated with a normoxic gas mixture. The venous cannula was kept open at the level of placenta to allow free outflow of the perfusate. All fetuses were then sacrificed by Thiopental overdose. The perfusion pressure was measured and recorded on both the maternal and fetal side by a PowerLab data acquisition system (ADInstruments, Spechbach, Germany).

After 15 min of stabilization, the Krebs solution perfusate was replaced with heparinized whole blood, kept at 37 ºC. Throughout the whole experiment, blood was continuously bubbled with 21 % O_2, 5 % CO_2 and 74 % N_2 and mixed in the reservoir. Samples for pH, pO_2 and pCO_2 measurement (ABL 5, Radiometer, Copenhagen, Denmark) were taken from the reservoir. The blood for perfusion (cca 15–25 ml), was obtained by cardiac punction into preheparinized syringes from adult rat males under deep ether anesthesia. After 10 min of blood perfusion, the resistance of the fetoplacental vasculature was assessed by measuring the perfusion pressure at the basal flow rate of 1 ml/min in both groups. To more precisely assess the resistive properties of the fetoplacental vascular bed, the relationship between perfusion pressure and flow was measured over a range of flow rates (P/Q ramp). To do so, the perfusion was stopped for 5–10 s and then the perfusion flow was increased in a ramp-like fashion from 0 to 2 ml/min in 120 s while measuring the perfusion pressure. At the end of each experiment, the mothers, fetuses and placentas were weighted and the weights of perfused and nonperfused placentas were compared.

Data were analyzed statistically using the StatView 5.0.1 software (SAS Institute, Cary, NC, USA) and presented as the means ± SEM. The unpaired t-test was used for inter-group comparisons, while paired t-test was used for intra-group comparisons. The P/Q ramps were analyzed using a linear regression for each preparation and its parameters (slope and pressure axis intercept) were compared between the groups using the unpaired t-test. $p < 0.05$ was considered statistically significant.

Results

As expected, the mothers and fetuses exposed to 7 days of hypoxia had significantly lower body weights than normoxic controls. The weights of the placentas after completing the protocol of artificial perfusion did not differ from the placentas from the same mother that were not used for artificial perfusion ($p = 0.791$ in normoxic group, $p = 0.071$ in hypoxic group) (Tab. 1). The perfusion protocol therefore did not result in gross edema of the preparation.

There were no statistically significant differences between the normoxic and hypoxic group in hematocrit, pH, pO_2 and pCO_2 of the perfusing blood (Tab. 2).

During the initial period of perfusion with Krebs solution, the baseline fetoplacental perfusion pressure (at the flow rate of 1 ml/min) was significantly higher in the chronically hypoxic group (34 ± 2 mmHg) than in the normoxic control group (23 ± 2 mmHg, $p < 0.01$) (Fig. 1). As expected, replacing this low-viscosity perfusate with full blood markedly increased the baseline fetoplacental perfusion pressure in both groups, and the difference between the normoxic (77 ± 5 mmHg) and chronically hypoxic group (94 ± 6 mmHg, $p < 0.05$) (Fig. 1) was somewhat larger than during the perfusion with salt solution (11 vs 17 mmHg).

During the brief suspension of perfusion before the P/Q ramp measurement, perfusion pressure reached a stable, positive value that did not differ between the groups (22 ± 3 mmHg in normoxic vs. 17 ± 3 mmHg in hypoxic group, $p = 0.30$).

As can be seen from Figure 2, the perfusion pressures were indistinguishable between the groups at the low flow rates during the P/Q ramp, up to ~ 0.8 ml/min. Above this flow rate, the perfusion pressure rose more sharply with increasing flow rate in the hypoxic than in the normoxic control group. Nevertheless, the P/Q ramp data fit well into a linear regression model in both groups – the linear regression coefficient was 0.91 ± 0.03 in the normoxic and 0.94 ± 0.01 in the chronically hypoxic group. The slope of the linear regression of the P/Q ramps was significantly higher in

Tab. 1. Maternal, placental and fetal weights.

Group	Maternal body weight (g)	Wet weight of placentas used for perfusion (mg)	Wet weight of placentas not used for perfusion (mg)	Fetal weight (g)
Normoxic	430±17	504±13	498±15	3.9±0.1
Hypoxic	338±12	549±16	506±12	3.2±0.1
P values (normoxic vs. hypoxic)	< 0.001	0.093	0.723	< 0.001

Tab. 2. Values of hematocrit, pH, pO_2 and pCO_2 in the perfusate.

Group	hematocrit	pH	pCO_2 (mmHg)	pO_2 (mmHg)
Normoxic	47±2	7.38±0.02	38±3	113±11
Hypoxic	45±1	7.42±0.04	38±4	94±5
P values (normoxic vs. hypoxic)	0.3372	0.3857	0.9198	0.2069

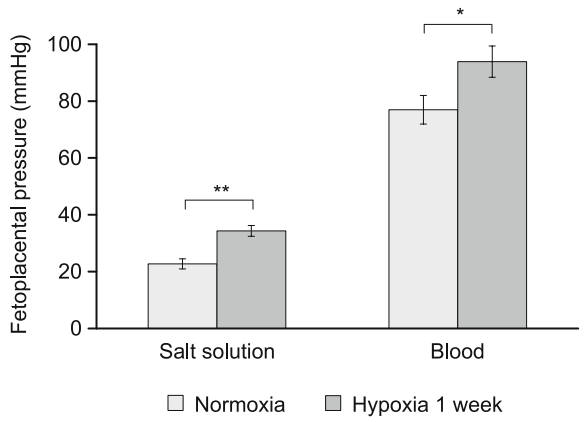

Fig. 1. Chronic hypoxia significantly increases fetoplacental perfusion pressure (mean ± SEM) in isolated rat placenta during perfusion at a constant flow rate of 1ml/min with Krebs solution (left) or whole blood (right). ** p < 0.01, * p < 0.05.

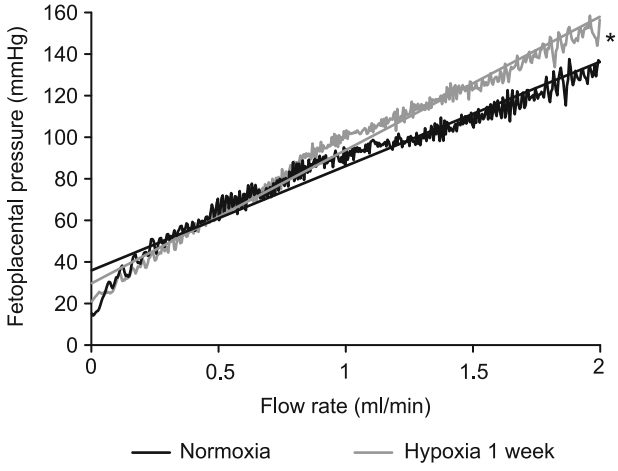

Fig. 2. Perfusion pressure-flow relationships in the fetal side of rat placenta. The tracings are averaged ramp measurements for each group, while lines are their linear regressions. * p < 0.05 for slopes of the regression lines

the chronically hypoxic compared to the normoxic group (0.29 ± 0.01 vs. 0.24 ± 0.01 mmHg/ml/min, p < 0.05, Fig. 2). The pressure axis intercept did not differ between the groups (normoxic group 36 ± 8 mmHg vs. hypoxic group 30 ± 5 mmHg, p = 0.49).

Discussion

This study showed that hypoxia during the last third of gravidity in rats resulted in an elevated vascular resistance on the fetal side of the placenta perfused with blood. This supports and extends our previous report of a similar effect in the rat placenta perfused with Krebs solution (8). While the difference in perfusion pressure between the groups in absolute terms (mmHg) was increased by replacing the low-viscosity Krebs solution perfusate with much higher viscous blood, the relative increase (% of the control group) was actually smaller. The rat placenta is a surprisingly useful model of human placental pathologies because of their numerous similarities (12). However, it is evident that perfusion with blood reflects the clinically relevant situation better than perfusion with Krebs solution. Nevertheless, our results showed that for studying the effects of hypoxia on the fetoplacental vascular resistance, it is not necessary to use the technically somewhat more difficult (and more demanding for the usage of experimental animals) model of blood-perfused placenta; the effects of hypoxia are sufficiently revealed in the model of Krebs-perfused placenta.

We used blood from adult donors, rather than fetal blood that perfuses the fetoplacental vessels in vivo. Using adult blood, however, should not confuse the results, because the whole fetal blood viscosity is comparable to that of adults, despite the somewhat higher fetal hematocrit. This is so mainly because of the lower plasma viscosity in the fetus (13, 14).

The possible mechanisms of action of chronic hypoxia on the fetoplacental vessels have been only minimally explored. What is quite clear is that an increased tension of the fetoplacental vascular smooth muscle is only little involved. Jakoubek et al (8) showed that a high dose of a potent vasodilator (sodium nitroprusside) was unable to remove the elevation of the fetoplacental vascular resistance induced by chronic maternal hypoxia. We have shown recently that fetoplacental vascular resistance is reduced only partially by another potent vasodilator, Rho-kinase inhibitor fasudil. After fasudil, the resistance remained significantly elevated in the hypoxic as compared to the normoxic control group (unpublished data). However, it is possible that the vasomotor component of the increased resistance could be higher in the in vivo conditions, because our measurements were performed under normoxic conditions (e.g., the pO_2 was higher than that in utero). Since hypoxia does cause fetoplacental vasoconstriction (15, 16), it is possible that under the hypoxic conditions in utero the vasoconstrictive component might be greater.

Since vasoconstriction is unlikely to be the major cause of the elevated resistance that we have observed, morphological remodeling of fetoplacental vasculature in chronic hypoxia (similar to that in the pulmonary circulation) remains as the alternative explanation. Indeed, morphological changes in the rat fetoplacental vasculature expected to result in an increased resistance, have been recently reported (17).

The increase in fetoplacental vascular resistance in chronic hypoxia was due to an increase in the slope of the P/Q lines rather than to a parallel shift (i.e. increased pressure axis intercept). This results appears to be in agreement with the study of Jakoubek et al (8) in which, however, the P/Q relationship was measured differently: not as a ramp, but rather as a series of distinct stepwise increases in the flow rate. The differences between the groups in that study were evaluated by repeated-measures ANOVA, so the slope and pressure-axis intercept were not determined. Nevertheless, the shape and relative position of the P/Q in normoxic and chronically hypoxic group appeared to be similar as in the present study, even though, of course, the each level of flow corresponded to lower pressures in the Krebs-perfused (8) than blood-perfused (the present study) placentas.

While the physiological interpretation of the P/Q slope and pressure-axis intercept in the placenta has not been elaborated, the analogy from other vascular beds (18) suggests that the increased P/Q slope may reflect a decreased vascular distensibility or smaller cross-sectional area of the fetoplacental vessels.

One cave of our study was that we did not achieve pO_2 levels similar to those in the fetus in utero (19). However this should blunt rather than augment any differences in fetoplacental vascular resistance between chronically hypoxic and normoxic groups. In the in vivo conditions (at significantly lower pO_2), the difference between normoxic and chronically hypoxic placentas might be expected to be even higher because of the presence of hypoxic vasoconstriction in the chronically hypoxic group. We have reported hypoxic fetoplacental vasoconstriction to be potentiated by chronic hypoxia (8). Chronic in utero hypoxia also elicits fetal polycythemia (20, 21) that is also expected to augment the difference between the hypoxic and control animals as compared to our situation of perfusion with normocytic blood of adult donors.

In summary, the study showed that chronic hypoxia during the last third of gestation resulted in a significant increase of the fetoplacental vascular resistance in the isolated, blood-perfused rat placenta. As hypothesized, the difference in perfusion pressure between the control and chronically hypoxic group was higher with blood perfusion than when the lower viscosity Krebs solution was used. Nevertheless, when this difference is expressed as percentage of the pressure in the control group, it is actually smaller in the blood-perfused than Krebs-perfused preparations. Thus, using somewhat more complicated blood perfusion does not confer any clear advantage for studying the effects of hypoxia on the fetoplacental vasculature over the simpler Krebs perfusion. It is important to stress that the 22 % increase in fetoplacental vascular resistance caused by chronic maternal hypoxia will reduce placental perfusion to the same extent (unless the vascular resistance in the fetal body also increases), which will result in considerable fetal undernutrition and presumably growth restriction, with all its known neonatal and long-term detrimental consequences.

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