Chronic hypoxia increases fetoplacental vascular resistance and vasoconstrictor reactivity in the rat

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An increase in fetoplacental vascular resistance caused by hypoxia is considered one of the key factors of placental hypoperfusion and fetal undernutrition leading to intrauterine growth restriction (IUGR), one of the serious problems in current perinatology. However, although acute hypoxia has been shown to cause fetoplacental vasoconstriction, the effects of more sustained hypoxic exposure are unknown. This study was designed to test the hypothesis that chronic hypoxia elicits elevations in fetoplacental resistance, that this effect is not completely reversible by acute reoxygenation, and that it is accompanied by increased acute vasoconstrictor reactivity of the fetoplacental vasculature. We measured fetoplacental vascular resistance as well as acute vasoconstrictor reactivity in isolated perfused placentae from rats exposed to hypoxia (10% O2) during the last week of a 3-wk pregnancy. We found that chronic hypoxia shifted the relationship between perfusion pressure and flow rate toward higher pressure values (by ~20%). This increased vascular resistance was refractory to a high dose of sodium nitroprusside, implying the involvement of other factors than increased vascular tone. Chronic hypoxia also increased vasoconstrictor responses to angiotensin II (by ~75%) and to acute hypoxic challenges (by >150%). We conclude that chronic prenatal hypoxia causes a sustained elevation of fetoplacental vascular resistance and vasoconstrictor reactivity that are likely to produce placental hypoperfusion and fetal undernutrition in vivo.

perfusion; pressure-flow relationship; hypoxic fetoplacental vasoconstriction

INTRAUTERINE GROWTH RESTRICTION (IUGR) is a serious problem of current perinatology (6). In addition to immediate prenatal and neonatal problems, small for gestational age children suffer increased risk of health problems throughout their life, including glycemic dysregulation, hypertension (both systemic and pulmonary), chronic lung disease, and coronary heart disease (for a review, see Ref. 6).

The causes and mechanisms of IUGR development are incompletely characterized. Chronic hypoxia of placental vessels leading to reduced placental perfusion is commonly considered one of the important factors (15, 46, 49, 51). Placental hypoxia could be caused by maternal hypoperfusion of the placenta due to local vascular disease or by generalized maternal hypoxia due to chronic lung or cardiovascular disease or high-altitude exposure (40, 45, 62). It is therefore somewhat surprising that the effects of chronic hypoxia on fetoplacental vascular resistance have never been documented. A few studies, including our own, have demonstrated that acute hypoxia (minutes) elicits reversible fetoplacental vasoconstriction (7, 8, 20, 27, 29, 50). However, the effects of more prolonged hypoxia (days to weeks) have not been studied at all.

In most organs, acute and chronic shortage of oxygen elicits more or less profound decreases in vascular resistance that help to deliver more oxygen to the tissues by increasing blood flow (23, 42, 60). The only exception known until recently is the pulmonary circulation, where acute hypoxia induces vasoconstriction that diverts blood flow from poorly toward better ventilated areas and thus optimizes blood oxygenation (42, 44). Chronic hypoxia causes sustained pulmonary hypertension, to which both increased vascular smooth muscle tension and structural remodeling of the vessel wall contribute (13, 41, 47, 55). The fetoplacental vasculature has been shown to resemble lung vessels in that it, too, responds to acute hypoxia by vasoconstriction (7, 8, 20, 27, 29, 50), presumably also in an attempt to divert blood flow to areas where it has a better chance for oxygenation (although the issue of the functional significance of the hypoxic vasoconstriction is more complicated in the placenta than in the lung) (26, 57). However, it is well known in the lung that pulmonary hypertension of chronic hypoxia is not a simple extension of acute hypoxic vasoconstriction. Instead, the fast acute response weakens after a while and is followed by a slowly developing pulmonary hypertension that has different mechanisms. This chronic hypoxic pulmonary hypertension persists to a large extent even during acute reoxygenation. Whether a similar situation exists in the placenta following the acute vasoconstrictor response to hypoxia is unknown. The first aim of the present study, therefore, was to test the hypothesis that chronic hypoxia causes a sustained elevation of vascular resistance in the fetal side of the placenta.

In addition to its effects on steady-state vascular resistance, chronic hypoxia is likely to affect reactivity to acute vasoactive stimuli. For example, acute exacerbations of hypoxia may have greater effects than similar decreases in oxygen availability suffered under otherwise normal conditions. For this reason, we also tested the hypothesis that chronic hypoxia increases fetoplacental vasoconstrictor reactivity to acute stimuli. Preliminary results have been reported as abstracts (30, 31).

METHODS

All procedures conformed to European Union regulations for experimental animal care and were approved by the ethical committee of the Second Faculty of Medicine, Charles University, Prague, Czech Republic.

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Pregnant Wistar rats (Biotest, Konárvice, Czech Republic) were exposed to hypoxia (10% O₂) in a normobaric hypoxic chamber (16, 19, 21, 24) during the last week of pregnancy (days 14–20 of gestation; term = 21 days). One day before the expected date of delivery, rats were removed from the chamber and used without delay to prepare the isolated, dually perfused placenta by a method adopted from Stulc et al. (48, 56). Briefly, under thiopental (Valeant, Praha, Czech Republic) anesthesia (50 mg/kg ip), the rat was placed in a bath of Ringer solution kept at 37°C (to keep the preparation temperature constant and physiological). The abdominal wall was open in midline, and a suitable placenta was then chosen by visual inspection of the uterus. Its supplying uterine artery was cannulated with a polyethylene 24-gauge catheter (Abbocath T, Abbot Ireland, Sligo, Ireland) perfused with Krebs saline (37°C) (all chemicals were obtained from Sigma, Prague, Czech Republic, unless noted otherwise) equilibrated with a normoxic gas mixture (bubbling with 21% O₂ + 5% CO₂ + 74% N₂ in a reservoir) at 1 ml/min. The segment of the uterus containing the chosen placenta and its fetus was separated by ligature from the rest of the organ. Uterine veins in this section were cut to allow a free outflow of the perfusate. The wall of the isolated uterine section was cut to deliver the fetus, which was then euthanized by an intraperitoneal thiopental overdose. The umbilical artery (the only one in the rat) and vein were quickly cannulated with 24-gauge polyethylene tubings to allow perfusion of the fetal side of the placenta with the same perfusate as the maternal side from a common reservoir at a constant flow rate of 1 ml/min. This flow rate was selected on the basis of preliminary experiments to result in perfusion pressures approximately corresponding to umbilical arterial mean pressure in vivo (~40–50 mmHg in the sheep) (5, 9, 10, 14). The distal end of the umbilical vein cannula (~3 cm) was left free at the level of the placenta to permit an easy outflow of the perfusate. It was used to take samples for measuring outflow pH and PO₂ (ABL 5, Radiometer, Copenhagen, Denmark). Perfusion pressure was recorded on the fetal side (and monitored on the maternal side) using a PowerLab data-acquisition system (ADInstruments, Spechbach, Germany). At the end of the perfusion, the wet weight of the perfused placenta was compared with that of other, nonperfused placentae from the same mother as an estimate of perfusion-induced edema.

Two separate experiments were performed. In the first experiment, we assessed the resistive properties of the fetoplacental vasculature by comparing the relationship between perfusion pressure and flow (P/Q) between the chronically hypoxic group (n = 6) and the normoxic control group (n = 6). After at least 15 min of preparation stabilization at a flow rate of 1 ml/min, we measured perfusion pressure during stepwise 0.2 ml/min increments of the fetoplacental flow rate (each step lasting ~2 min) ranging from 0 to 1.8 ml/min. To determine the contribution of active tension to the observed difference in P/Q between normoxic and hypoxic placentae, we then added a high dose of a potent vasodilator [sodium nitroprusside (SNP)] at a final perfusate concentration of 60 mM into the perfusate during the baseline flow rate of 1 ml/min and measured the P/Q relationship again.

In the second experiment, we investigated acute vasocostrictr reactivity of the fetoplacental vasculature during a constant fetoplacental flow of 1 ml/min. After at least 15 min of preparation stabilization, three increasing bolus doses of ANG II (0.1, 0.15, and 0.2 μg) were injected into the fetal arterial cannula at 10-min intervals. Acute hypoxic vasocostruction was then elicited by switching the bubbling of the perfusate to a 5% CO₂ + 95% N₂ mixture for 10–15 min. This acute hypoxic challenge was repeated once more after a 15-min normoxic interval. In this experiment, we compared nine placentae from control rats with six preparations isolated at the end of chronic hypoxia.

Results were evaluated statistically using StatView 5.0.1 software (SAS Institute, Cary, NC) and are presented as means ± SE. P/Q lines and reactivity data were compared between groups using repeated-measures ANOVA. An unpaired t-test was used for simple comparisons between the groups (maternal body weight, etc.). Weights of perfused and nonperfused placentae from the same mother were compared with a paired t-test. Differences were regarded as significant at P < 0.05.

RESULTS

As expected, chronic hypoxia reduced the body weight of both the mothers and fetuses. Placental weight was affected neither by chronic hypoxia nor by our perfusion protocol (Table 1).

In the first experiment, we observed a tendency for higher baseline fetoplacental perfusion pressure (at 1 ml/min flow rate) in the hypoxic group than in the control group (64 ± 3 vs. 53 ± 4 mmHg); however, the difference narrowly missed our predefined level of statistical significance (P = 0.056). However, the more precise characterization of the resistive properties by the P/Q analysis revealed that chronic hypoxia caused a significant shift of the P/Q relationship toward higher pressures by ~20% (Fig. 1A).

To measure the contribution of active vascular wall tension to the elevated fetoplacental vascular resistance in chronic hypoxia, P/Q lines were compared in each group before and after the administration of a high dose of a vasodilator (SNP). As expected, it had no effect in the normoxic group (Fig. 1B). Surprisingly, in the chronically hypoxic group, the P/Q lines also did not differ before and after SNP treatment (Fig. 1C), implying that chronic hypoxia does not cause persistent fetoplacental vasoconstriction. Since the action of SNP depends on the presence of functional endogenous enzymatic systems, the ability of SNP to indeed elicit vasodilation in this particular preparation was tested in a supplementary experiment. It showed that before SNP administration, ANG II injection increased the perfusion pressure by 15%. After SNP administration, ANG II caused a no more than 5% increase in perfusion pressure, confirming the ability of SNP to inhibit vascular tone in rat fetoplacental vessels in the last third of gestation.

In the second experiment aimed at the evaluation of vaso-reactivity, the baseline fetoplacental perfusion pressure (at 1 ml/min flow rate) was significantly higher in the chronically hypoxic group (48 ± 3 mmHg) than in the normoxic control group (36 ± 3 mmHg, P = 0.0048).

All three ANG II injections elicited rapid and transient peaks in perfusion pressure that returned virtually to baseline (>95% of baseline) within 10 min. ANG II challenge was repeated once more after a 15-min normoxic interval. Acute hypoxic vasoconstriction was then elicited by switching the perfusate to a 5% CO₂ + 95% N₂ mixture for 10–15 min.

P values (normoxic vs. hypoxic) 0.012 0.43 0.82

Table 1. Maternal, fetal, and placental weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight of Mothers, g</th>
<th>Wet Weight of Placenta After Perfusion, mg</th>
<th>Wet Weight of Nonperfused Placenta, mg</th>
<th>Fetal Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normoxic controls</td>
<td>426 ± 31</td>
<td>562 ± 151</td>
<td>647 ± 75</td>
<td>4.08 ± 0.05</td>
</tr>
<tr>
<td>1-wk hypoxia</td>
<td>323 ± 11</td>
<td>456 ± 54</td>
<td>675 ± 71</td>
<td>3.26 ± 0.16</td>
</tr>
<tr>
<td>P values (normoxic vs. hypoxic)</td>
<td>0.012</td>
<td>0.43</td>
<td>0.82</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SE. There were no significant differences in weights between perfused and nonperfused placentae (by paired t-test).
reversibility) within the recovery period of 10 min between the injections (Fig. 2). The magnitude of pressure responses was significantly larger in the chronically hypoxic group than in the normoxic control group (Fig. 3A). However, when the ANG II responses were expressed as a percentage of baseline perfusion pressure before the injection, the groups did not differ significantly (Fig. 3B). In the relatively narrow range of doses used, the magnitude of the responses was not dependent on the ANG II dose in either group (Fig. 3).

Acute hypoxic challenges reduced effluent PO2 equally in the normoxic group (from 117 ± 3 to 81 ± 2 mmHg) and chronically hypoxic group (from 119 ± 3 to 84 ± 3 mmHg). They elicited relatively slow (compared with ANG II) increases in perfusion pressure that were maintained throughout the whole challenge and returned to close to baseline (>85% reversibility) within the 15-min normoxic period between the challenges (Fig. 2). The magnitude of hypoxic responses of perfusion pressure was significantly greater in the chronically hypoxic group than in the normoxic control group (Fig. 4A). This was also true for responses expressed as a percentage of normoxic perfusion pressure before the hypoxic challenge (Fig. 4B). Responses of perfusion pressure to acute hypoxia were not significantly increased or diminished with the repetition of hypoxic challenges.

**DISCUSSION**

This study shows that chronic hypoxia elicits a sustained elevation of fetoplacental vascular resistance as well as increased acute vasoconstrictor reactivity. Such an effect has been implicitly assumed in the mechanism of IUGR and possibly also preeclampsia (15, 46, 49, 51), but its existence has not been previously documented. Therefore, our results fill in the missing evidence for a hypothesized chain of events in which elevation of fetoplacental vascular resistance by chronic hypoxia causes sustained placental hypoperfusion and consequently impaired fetal nutrition and growth that eventually manifests as IUGR. It is important to note that although the elevation of fetoplacental vascular resistance by chronic hypoxia resembles a similar phenomenon in the lung (whereas in all other vascular beds hypoxia tends to decrease resistance), a key difference between the two organs is that the functional result in vivo is an increase in arterial pressure in the lung (pulmonary hypertension), whereas reduced perfusion (at about the same pressure) can be expected in the placenta.

The term “chronic” is used here in a relative sense. In human medicine, it typically describes longer periods than those used here. However, in the context of pregnancy, 1 wk in our study represents one-third of the whole gestation period of the rat, roughly corresponding to the last trimester of human pregnancy. We chose this duration of hypoxia because at this time embryogenesis is completed and a fully functional placenta has already been formed. Thus, the results reflect the effect of hypoxia on already established fetoplacental vessels rather than on their initial formation. This does not exclude the possibility...
that hypoxia during the last third of pregnancy may affect the functional maturation of fetoplacental vessels.

An important aspect of our finding is that the elevation of fetoplacental vascular resistance caused by chronic hypoxia is sustained, i.e., it is refractory to acute reoxygenation. This is evident from the fact that it was observed in placentae perfused with solution equilibrated with 21% O₂. A high dose of SNP was unable to reduce vascular resistance in the hypoxic group.

Fig. 3. Chronic hypoxia potentiates fetoplacental vasoconstrictor reactivity to ANG II. The increases in perfusion pressure (during a constant flow rate) elicited by ANG II were higher in the hypoxic group than in the normoxic group (A). However, the responses to ANG II expressed in relation to baseline perfusion pressure before the ANG II injection were not significantly different between the groups (B). *P < 0.05 between the normoxic and chronically hypoxic group.

Fig. 4. Chronic hypoxia potentiates fetoplacental vasoconstrictor reactivity to acute hypoxic stimuli. The increases in perfusion pressure (during a constant flow rate) elicited by acute hypoxic challenges (repeated twice) were higher in the hypoxic group than in the normoxic group (A). This was also true when the responses to hypoxia were expressed in relation to normoxic baseline perfusion pressure before each hypoxic challenge (B). *P < 0.05 between the normoxic and chronically hypoxic group.
implying that elevated vascular wall tone does not contribute to the increased resistance under these conditions. Nevertheless, it is quite likely that a quickly reversible vasoconstrictive component is in fact present in situ when the hypoxic conditions are still present because hypoxia (at least acute hypoxia) is known to elicit reversible fetoplacental vasoconstriction (7, 8, 20, 27, 50) and this acute hypoxic vasoconstriction was elevated by chronic hypoxia in our study. This putative vasoconstrictor component might have been removed in our experiments by the normoxic conditions of the preparation, rendering the placenta unresponsive to SNP. This might also contribute to the fact that, although significant, the difference observed in normoxic control rats were relatively smaller than we have published previously in the isolated cotyledon of the human placenta (~12–14% of the baseline perfusion pressure in the rat vs. >20% in the human cotyledon) (20). It is important to note that not only is this study the first description of HFPV in the rat but also in the whole placenta (as opposed to isolated cotyledon in previous studies) and, more importantly, in a preparation not affected by the rather traumatic process of delivery. The perfused rat placenta thus proves a suitable model of HFPV for studies involving prior experimental manipulation that would not be technically or ethically feasible in humans (such as chronic hypoxic exposure).

The responses to ANG II were elevated by chronic hypoxia in proportion to preexisting resistance. In general, vasoconstrictor reactivity depends on resting vascular tone. This, however, was not elevated by chronic hypoxia in our preparation, as shown by our results with SNP. Our finding about ANG II reactivity could alternatively be explained by an increased amount of vascular smooth muscle as a reason for both increased resting resistance and ANG II responses. It remains to be elucidated whether such an increase (analogous to the situation in the lung) does indeed happen.

Although the reactivity to ANG II was elevated by chronic hypoxia only in absolute expression, this is still likely to have a functional impact. Due to this increased reactivity, abnormally high values of resistance could be reached when endogenous levels of vasoconstrictors such as ANG II are elevated, likely aggravating placental hypoperfusion and insufficient fetal nutrition and growth. However, in the case of the rat species, the magnitude of vasoconstrictor responses to ANG II was quite small, and thus any functional consequences are unlikely to be dramatic. Based on our results, ANG II appears as a suboptimal tool for studying rat fetoplacental vasoconstrictor reactivity.

Unlike the reactivity to ANG II, the acute HFPV was increased by chronic hypoxia both in absolute and relative terms. This implies that the mechanism of acute HFPV is upregulated by chronic hypoxia relatively selectively. The mechanism of HFPV includes inhibition of potassium channels (20), depolarization, activation of calcium influx through L-type channels (29), and possibly inhibition of nitric oxide signaling (7). As discussed above, these mechanisms are likely to be altered by chronic hypoxia of the placenta. Regardless of its mechanism, this hyperreactivity to acute hypoxia is likely to further aggravate placental hypoperfusion and impairment of fetal nutrition and growth during exacerbations of the chronic hypoxic state.

A methodological limitation of the present study that should be acknowledged is our inability to achieve (even during anoxic bubbling of the perfusate) effluent Po2 levels similar to those in the fetus in utero (arterial Po2 ~30 mmHg) (1, 2, 28, 34, 52–54). This is due to diffusion of oxygen through the
walls of perfusion tubings (which have to be small and thin for the rat placenta) and other surfaces. Breathing hypoxic mixtures (9–15% O₂) by the mother reduces fetal arterial Po₂ in vivo by 25–50% (1, 2, 28, 34, 52–54). In our experiments, the hypoxic challenges reduced Po₂ by 30%.

In conclusion, we found that chronic hypoxia increases fetoplacental vascular resistance and also vasoconstrictor reactivity to ANG II and acute hypoxic challenges. These changes are likely to result in fetal hypoperfusion of the placenta and consequently impairment in fetal nutrition and growth. This could explain IUGR found in cases of uterine hypoxia or hypoperfusion and possibly also some delayed effects of intrauterine hypoxia, such as a greater susceptibility to pulmonary hypertension in adulthood (17, 18). Further study of the mechanisms involved has the potential to bring therapeutically useful solutions.

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