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

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Jakoubek V, Bíbová J, Herget J, Hampl V. Chronic hypoxia increases fetoplacental vascular resistance and vasoconstrictor reactivity in the rat. *Am J Physiol Heart Circ Physiol* 294: H1638–H1644, 2008; First published February 29, 2008; doi:10.1152/ajpheart.01120.2007.— 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 neonatology. 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% O_2) 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 $\sim 20\%$). 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 $\sim 75\%$) 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.

perfused placenta; 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, Konárovice, Czech Republic) were exposed to hypoxia (10% O_2) 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 chemials were obtained from Sigma, Prague, Czech Republic, unless noted otherwise) equilibrated with a normoxic gas mixture (bubbling with 21% O_2 + 5% CO_2 + 74% N_2 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 uterus 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 Po_2 (ABL 5, Radiometer, Copenhagen, Denmark). Perfusion pressure was recorded on the fetal side (and monitored on the maternal side) using a PowerLab data-aqusition 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 vasoconstrictor 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 vasoconstriction was then elicited by switching the bubbling

of the perfusate to a 5% CO_2 + 95% N_2 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. 1*A*).

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. 1*B*). Surprisingly, in the chronically hypoxic group, the P/Q lines also did not differ before and after SNP treatment (Fig. 1*C*), 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 vasoreactivity, 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%

Table 1. *Maternal, fetal, and placental weights*

Group	Body Weight of Mothers, g	Wet Weight of Placentae After Perfusion, mg	Wet Weight of Nonperfused Placentae, mg	Fetal Weight, g
Normoxic controls	426±31	562±151	647±75	4.08±0.05
1-wk hypoxia	323±11	456±54	675±71	3.26±0.16
P values (normoxic vs. hypoxic)	0.012	0.43	0.82	<0.0001

Data are means ± SE. There were no significant differences in weights between perfused and nonperfused placentae (by paired *t*-test).
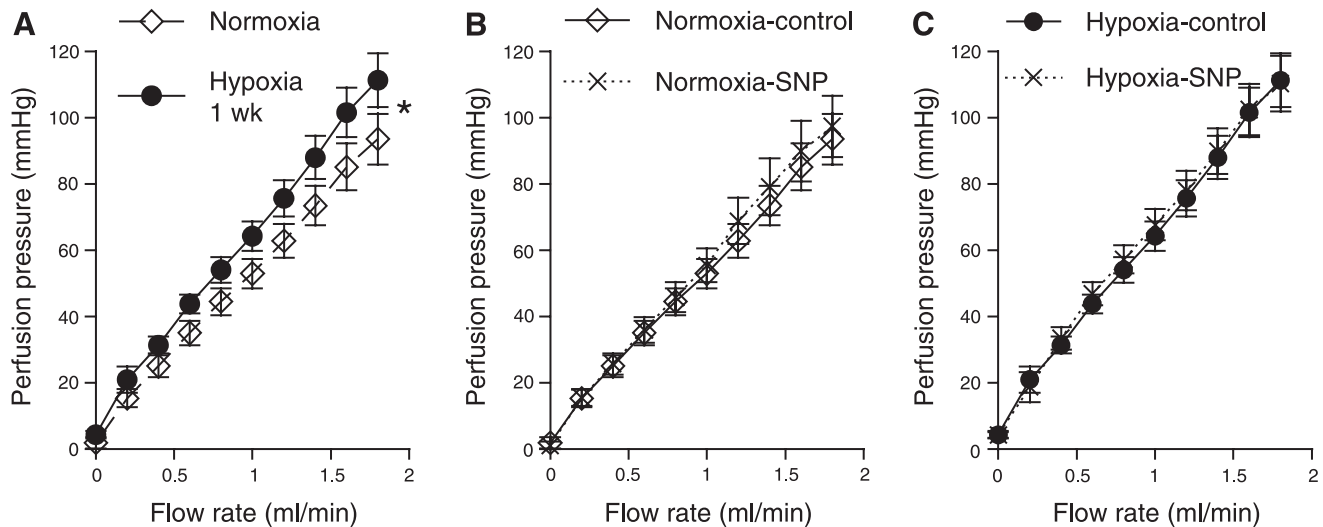
Fig. 1. Perfusion pressure-flow relationship in the fetal side of the rat placenta. The pressure-flow line was shifted toward significantly higher pressures in the hypoxic group (*A*). Sodium nitroprusside (SNP) had no effect on the pressure-flow line in both the normoxic group (*B*) and the hypoxic group (*C*). The entire pressure-flow lines differed between the normoxic and chronically hypoxic groups (by repeated-measures ANOVA) in *A* (*$P < 0.05$).

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. 3*A*). 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. 3*B*). 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 P_{O_2} 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
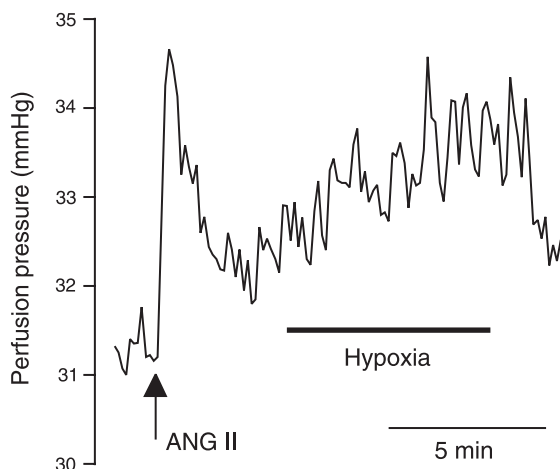

Fig. 2. ANG II and acute hypoxia elicit reversible vasoconstriction in the isolated, double-perfused rat placenta. A typical example of a perfusion pressure response to an ANG II bolus (0.2 μg) and to an acute hypoxic stimulus (change of perfusate bubbling from 21% to 0% O_2) during constant flow rate (1 ml/min) perfusion in a control placenta is shown.

perfusion pressure was significantly greater in the chronically hypoxic group than in the normoxic control group (Fig. 4*A*). This was also true for responses expressed as a percentage of normoxic perfusion pressure before the hypoxic challenge (Fig. 4*B*). 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
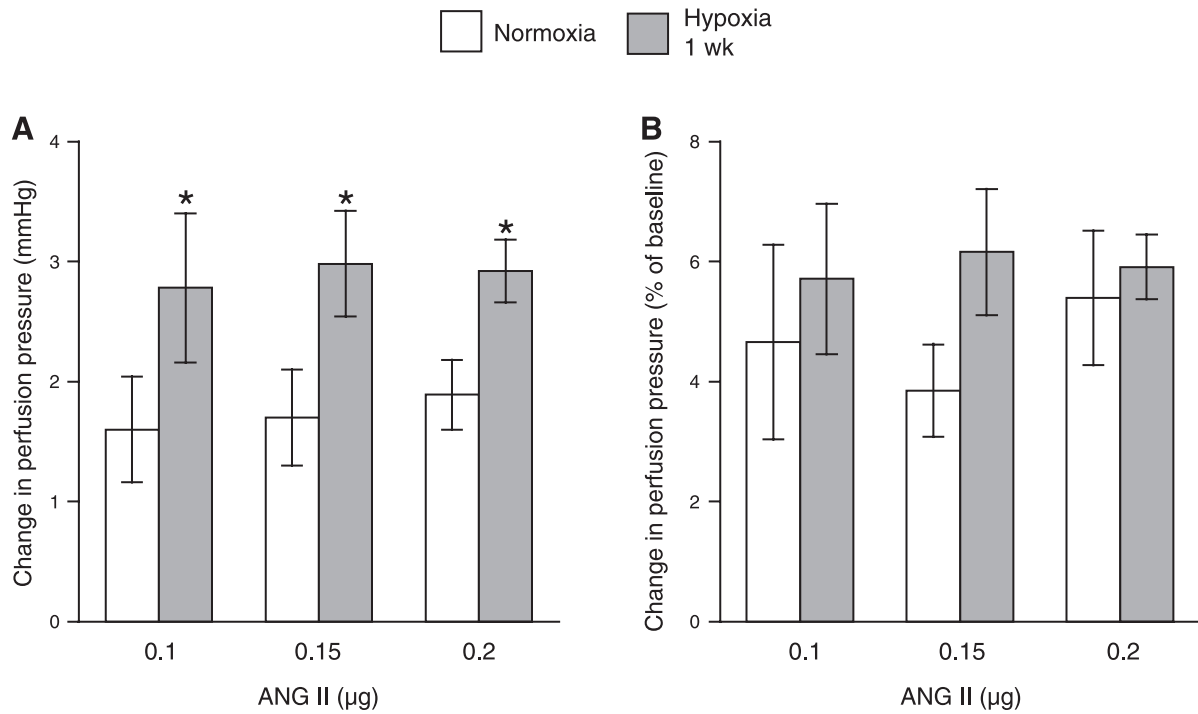
Fig. 3. Chronic hypoxia potentiates fetoplacental vasoconstrictor reactivity to ANG II. The increases in perfusion pressure (during a constant flow rate) elicited by ANG II were higher in the hypoxic group than in the normoxic group (*A*). However, the responses to ANG II expressed in relation to baseline 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.

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% O_2. A high dose of SNP was unable to reduce vascular resistance in the hypoxic group,
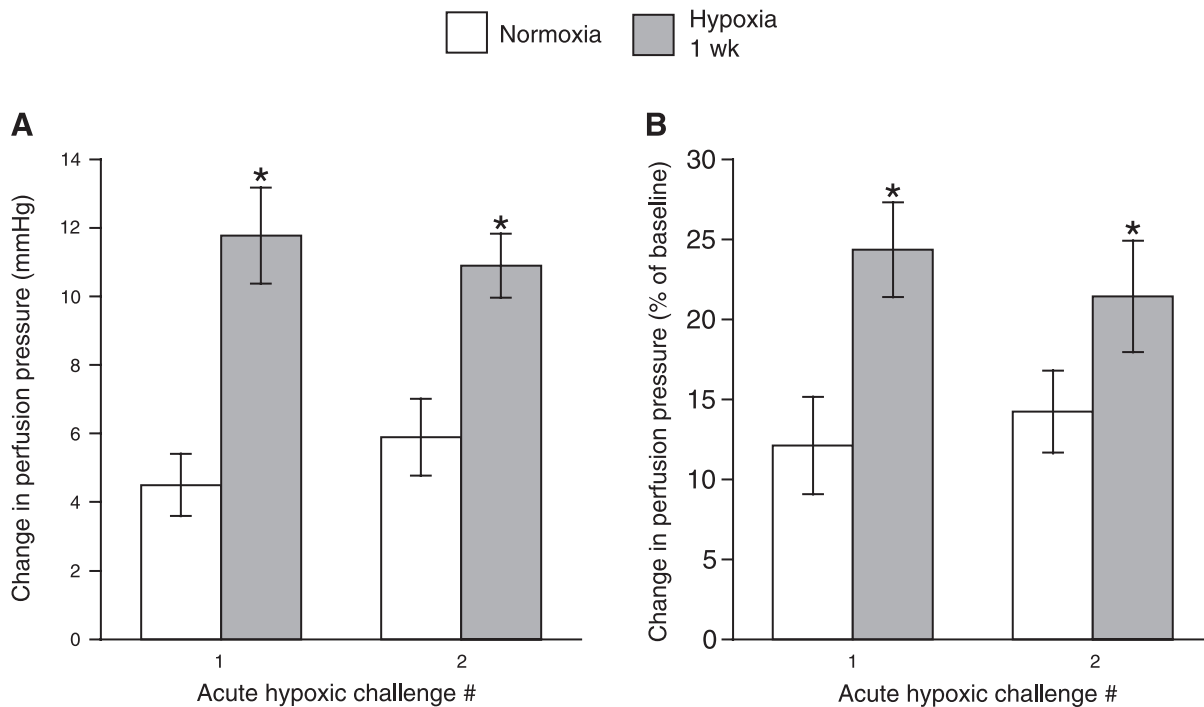

Fig. 4. Chronic hypoxia potentiates fetoplacental vasoconstrictor reactivity to acute hypoxic stimuli. The increases in perfusion pressure (during a constant flow rate) elicited by acute hypoxic challenges (repeated twice) were higher in the hypoxic group than in the normoxic group (*A*). This was also 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 between the chronically hypoxic and normoxic group was not very large (by ~20–25%). We assume that another reason for this was the very low viscosity of the perfusate (no blood cells and no albumin), so that the resistance was low and thus also its changes had to be numerically relatively small. In any case, our data show that chronic hypoxia causes an increase in fetoplacental vascular resistace, of which at least a portion is resistant to acute reoxygenation and to vasodilator administration. This suggests that morphological remodeling of fetoplacental vessels might be the cause of the increased resistance in chronic hypoxia.

While the effects of chronic hypoxia on the morphology of uteroplacental vessels are quite well described (for a review, see Ref. 43), morphological changes induced in the fetoplacental vasculature by chronic hypoxia have been surprisingly little studied. According to Zamudio (62), "the available literature on high altitude placentae derives from less than 100 pregnancies." Chronic hypoxia of high altitude results in increased villous vascularization, greater villus capillary diameter, thinning of the villous membranes, and proliferation of villous cytotrophoblasts (11, 33, 58, 62). In addition, Khalid et al. (33) described an elevated incidence of syncytial knots, also known from pregnancies with hypertension, diabetes, and preeclampsia. Most of these changes would be expected to reduce, rather than increase, resistance at the capillary level. However, the increase in overall resistance observed in our study is likely to involve resistance arterioles, and their changes in chronic hypoxia are currently unknown. In chronic hypoxic pulmonary hypertension, the lumen of resistive "arterioles" decreases despite a rise in capillarity (13, 41).

This study did not directly investigate possible mechanisms of the chronic hypoxic elevation of fetoplacental vascular resistance. However, it is likely that factors involved in the analogous chronic hypoxic pulmonary hypertension would also play some role in the placenta. These include activation of the Rho kinase system (12, 47, 55), cell membrane depolarization due to alterations in the activity of various potassium channels (19, 25, 38), oxidant injury of the vascular wall by oxygen radicals (35, 37) and perhaps nitric oxide (3, 16, 22), subsequent extracellular matrix accumulation (24, 61) mediated partly by mast cells (59), and alterations in serotonin signaling (4, 32, 36, 39). Because of the putative practical relevance of this phenomenon, further research of these mechanisms is warranted.

Another novel finding of this study is the increased fetoplacental vasoconstrictor reactivity in chronic hypoxia. This was true for absolute magnitudes of responses to ANG II and for both absolute and relative magnitudes of responses to acute hypoxia. As for acute hypoxic fetoplacental vasoconstriction

(HFPV), this is the first study to document its existence in other species than humans. Qualitatively, the response (measured as a change in perfusion pressure at a constant flow rate) looks about the same in our preparation as in the perfused cotyledon of the human placenta, where it has been described repeatedly (7, 8, 20, 27, 50). In both preparations, a slow rise in pressure (over several minutes) is followed by a more or less stable plateau and an almost complete reversal of the response within minutes of reoxygenation (Fig. 2). Quantitativly, the responses 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 P_{O_2} levels similar to those in the fetus in utero (arterial P_{O_2} ~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% O_2) by the mother reduces fetal arterial P_{O_2} in vivo by 25–50% (1, 2, 28, 34, 52–54). In our experiments, the hypoxic challenges reduced P_{O_2} 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.

REFERENCES

1. **Abman SH, Accurso FJ, Wilkening RB, Meschia G.** Persistent fetal pulmonary hypoperfusion after acute hypoxia. *Am J Physiol Heart Circ Physiol* 253: H941–H948, 1987.
2. **Alonso JG, Okai T, Longo LD, Gilbert RD.** Cardiac function during long-term hypoxemia in fetal sheep. *Am J Physiol Heart Circ Physiol* 257: H581–H589, 1989.
3. **Archer S, Hampl V, McKenzie Z, Nelson D, Huang J, Shultz P, Weir EK.** Role of endothelial-derived nitric oxide in normal and hypertensive pulmonary vasculature. *Semin Respir Med* 15: 179–189, 1994.
4. **Belohlávková S, Simák J, Kokesová A, Hnilicková O, Hampl V.** Fenfluramine-induced pulmonary vasoconstriction: role of serotonin receptors and potassium channels. *J Appl Physiol* 91: 755–761, 2001.
5. **Berman W, Goodlin RC, Heymann MA, Rudolph AM.** Relationship between pressure and flow in the umbilical and uterine circulations of the sheep. *Circ Res* 38: 262–266, 1976.
6. **Brodsky D, Christou H.** Current concepts in intrauterine growth restriction. *J Intens Care Med* 19: 307–319, 2004.
7. **Byrne BM, Howard RB, Morrow RJ, Whiteley KJ, Adamson SL.** Role of the L-arginine nitric oxide pathway in hypoxic fetoplacental vasoconstriction. *Placenta* 18: 627–634, 1997.
8. **Challis DE, Pfarrer CD, Ritchie JWK, Koren G, Adamson SL.** Glucose metabolism is elevated and vascular resistance and maternofetal transfer is normal in perfused placental cotyledons from severely growth-restricted fetuses. *Pediatr Res* 47: 309–315, 2000.
9. **Edwards LJ, McMillen IC.** Maternal undernutrition increases arterial blood pressure in the sheep fetus during late gestation. *J Physiol* 533: 561–570, 2001.
10. **Edwards LJ, Simonetta G, Owens JA, Robinson JS, McMillen IC.** Restriction of placental and fetal growth in sheep alters fetal blood pressure responses to angiotensin II and captopril. *J Physiol* 515: 897–904, 1999.
11. **Espinoza J, Sebire N, McAuliffe F, Krampl E, Nicolaides K.** Placental villus morphology in relation to maternal hypoxia at high altitude. *Placenta* 22: 606–608, 2001.
12. **Fagan KA, Oka M, Bauer NR, Gebb SA, Ivy DD, Morris KG, McMurtry IF.** Attenuation of acute hypoxic pulmonary vasoconstriction and hypoxic pulmonary hypertension in mice by inhibition of Rho-kinase. *Am J Physiol Lung Cell Mol Physiol* 287: L656–L664, 2004.
13. **Farber HW, Loscalzo J.** Pulmonary arterial hypertension. *N Engl J Med* 351: 1655–1665, 2004.
14. **Forhead AJ, Pipkin FB, Fowden AL.** Effect of cortisol on blood pressure and the renin-angiotensin system in fetal sheep during late gestation. *J Physiol* 526: 167–176, 2000.
15. **Fowden AL, Giussani DA, Forhead AJ.** Intrauterine programming of physiological systems: causes and consequences. *Physiology* 21: 29–37, 2006.
16. **Hampl V, Bíbová J, Banasová A, Uhlík J, Miková D, Hnilicková O, Lachmanová V, Herget J.** Pulmonary vascular iNOS induction participates in the onset of chronic hypoxic pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 290: L11–L20, 2006.
17. **Hampl V, Bíbová J, Herget J.** Perinatal history of hypoxia leads to lower vascular pressures and hyporeactivity to angiotensin II in isolated lungs of adult rats. *Physiol Res* 49: 567–575, 2000.
18. **Hampl V, Bíbová J, Ostádalová I, Povysilová V, Herget J.** Gender differences in the long-term effects of perinatal hypoxia on the pulmonary circulation in rats. *Am J Physiol Lung Cell Mol Physiol* 285: L386–L392, 2003.
19. **Hampl V, Bíbová J, Povysilová V, Herget J.** Dehydroepiandrosterone sulfate reduces experimental pulmonary hypertension in rats. *Eur Respir J* 21: 862–865, 2003.
20. **Hampl V, Bíbová J, Straňák Z, Wu X, Michelakis ED, Hashimoto K, Archer SL.** Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition. *Am J Physiol Heart Circ Physiol* 283: H2440–H2449, 2002.
21. **Hampl V, Herget J.** Perinatal hypoxia increases hypoxic pulmonary vasoconstriction in adult rats recovering from chronic exposure to hypoxia. *Am Rev Respir Dis* 142: 619–624, 1990.
22. **Hampl V, Herget J.** Role of nitric oxide in the pathogenesis of chronic pulmonary hypertension. *Physiol Rev* 80: 1337–1372, 2000.
23. **Hampl V, Weir EK, Archer SL.** Endothelium-derived nitric oxide is less important for basal tone regulation in the pulmonary than the renal vessels of adult rat. *J Vasc Med Biol* 5: 22–30, 1994.
24. **Herget J, Novotná J, Bíbová J, Povysilová V, Vanková M, Hampl V.** Metalloproteinase inhibition by Batimastat attenuates pulmonary hypertension in chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol* 285: L199–L208, 2003.
25. **Hong Z, Weir EK, Nelson DP, Olschewski A.** Subacute hypoxia decreases voltage-activated potassium channel expression and function in pulmonary artery myocytes. *Am J Respir Cell Mol Biol* 31: 337–343, 2004.
26. **Howard R.** Control of human placental blood flow. *Med Hypotheses* 23: 51–58, 1987.
27. **Howard RB, Hosokawa T, Maguire H.** Hypoxia-induced fetoplacental vasoconstriction in perfused human placental cotyledons. *Am J Obstet Gynecol* 157: 1261–1266, 1987.
28. **Jackson BT, Piasecki GJ, Novy MJ.** Fetal responses to altered maternal oxygenation in rhesus monkey. *Am J Physiol Regul Integr Comp Physiol* 252: R94–R101, 1987.
29. **Jakoubek V, Bíbová J, Hampl V.** Voltage-gated calcium channels mediate hypoxic vasoconstriction in the human placenta. *Placenta* 27: 1030–1033, 2006.
30. **Jakoubek V, Bíbová J, Venclíková K, Hampl V.** Chronic hypoxia elevates fetoplacental vascular resistance (Abstract). *FASEB J* 20: A1217, 2006.
31. **Jakoubek V, Bíbová J, Venclíková K, Hampl V.** Chronic hypoxia increases fetoplacental vascular reactivity (Abstract). *FASEB J* 21: A1287, 2007.
32. **Keegan A, Morecroft I, Smillie D, Hicks MN, MacLean MR.** Contribution of the 5-HT$_{1B}$ receptor to hypoxia-induced pulmonary hypertension: converging evidence using 5-HT$_{1B}$-receptor knockout mice and the 5-HT$_{1B/1D}$-receptor antagonist GR127935. *Circ Res* 89: 1231–1239, 2001.
33. **Khalid M, Ali M, Ali K.** Full-term birth weight and placental morphology at high and low altitude. *Int J Gynaecol Obstet* 57: 259–265, 1997.
34. **Kitanaka T, Alonso JG, Gilbert RD, Siu BL, Clemons GK, Longo LD.** Fetal responses to long-term hypoxemia in sheep. *Am J Physiol Regul Integr Comp Physiol* 256: R1348–R1354, 1989.
35. **Lachmanová V, Hnilicková O, Povysilová V, Hampl V, Herget J.** N-acetylcysteine inhibits hypoxic pulmonary hypertension most effectively in the initial phase of chronic hypoxia. *Life Sci* 77: 175–182, 2005.
36. **Launay JM, Hervé P, Peoc'h K, Tournois C, Callebert J, Nebigil CG, Etienne N, Drouet L, Humbert M, Simonneau G, Maroteaux L.** Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. *Nat Med* 8: 1129–1135, 2002.

37. **Liu JQ, Zelko IN, Erbynn EM, Sham JS, Folz RJ.** Hypoxic pulmonary hypertension: role of superoxide and NADPH oxidase (gp91phox). *Am J Physiol Lung Cell Mol Physiol* 290: L2–L10, 2006.

38. **Mandegar M, Yuan JX.** Role of K^+ channels in pulmonary hypertension. *Vasc Pharmacol* 38: 25–33, 2002.

39. **Marcos E, Adnot S, Pham MH, Nosjean A, Raffestin B, Hamon M, Eddahibi S.** Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. *Am J Respir Crit Care Med* 168: 487–493, 2003.

40. **Mateev S, Sillau AH, Mouser R, McCullough RE, White MM, Young DA, Moore LG.** Chronic hypoxia opposes pregnancy-induced increase in uterine artery vasodilator response to flow. *Am J Physiol Heart Circ Physiol* 284: H820–H829, 2003.

41. **McLaughlin VV, McGoon MD.** Pulmonary arterial hypertension. *Circulation* 114: 1417–1431, 2006.

42. **Michiels C.** Physiological and pathological responses to hypoxia. *Am J Pathol* 164: 1875–1882, 2004.

43. **Moore LG.** Fetal growth restriction and maternal oxygen transport during high altitude pregnancy. *High Alt Med Biol* 4: 141–156, 2003.

44. **Moudgil R, Michelakis ED, Archer SL.** Hypoxic pulmonary vasoconstriction. *J Appl Physiol* 98: 390–403, 2005.

45. **Murphy VE, Gibson PG, Smith R, Clifton VL.** Asthma during pregnancy: mechanisms and treatment implications. *Eur Respir J* 25: 731–750, 2005.

46. **Myatt L.** Control of vascular resistance in the human placenta. *Placenta* 13: 329–341, 1992.

47. **Nagaoka T, Morio Y, Casanova N, Bauer N, Gebb S, McMurtry I, Oka M.** Rho/Rho kinase signaling mediates increased basal pulmonary vascular tone in chronically hypoxic rats. *Am J Physiol Lung Cell Mol Physiol* 287: L665–L672, 2004.

48. **Pavek P, Staud F, Fendrich Z, Sklenarova H, Libra A, Novotna M, Kopecky M, Nobilis M, Semecky V.** Examination of the functional activity of P-glycoprotein in the rat placental barrier using rhodamine 123. *J Pharmacol Exp Ther* 305: 1239–1250, 2003.

49. **Poston L, McCarthy AL, Ritter JM.** Control of vascular resistance in the maternal and feto-placental arterial beds. *Pharmacol Ther* 65: 215–239, 1995.

50. **Ramasubramanian R, Johnson RF, Downing JW, Minzter BH, Paschall RL.** Hypoxemic fetoplacental vasoconstriction: a graduated response to reduced oxygen conditions in the human placenta. *Anesth Analg* 103: 439–442, 2006.

51. **Reynolds LP, Caton JS, Redmer DA, Grazul-Bilska AT, Vonnahme KA, Borowicz PP, Luther JS, Wallace JM, Wu G, Spencer TE.** Evidence for altered placental blood flow and vascularity in compromised pregnancies. *J Physiol* 572: 51–58, 2006.

52. **Rurak DW, Richardson BS, Patrick JE, Carmichael L, Homan J.** Blood flow and oxygen delivery to fetal organs and tissues during sustained hypoxemia. *Am J Physiol Regul Integr Comp Physiol* 258: R1116–R1122, 1990.

53. **Rurak DW, Richardson BS, Patrick JE, Carmichael L, Homan J.** Oxygen consumption in the fetal lamb during sustained hypoxemia with progressive acidemia. *Am J Physiol Regul Integr Comp Physiol* 258: R1108–R1115, 1990.

54. **Sherman DJ, Ross MG, Day L, Humme J, Ervin MG.** Fetal swallowing: response to graded maternal hypoxemia. *J Appl Physiol* 71: 1856–1861, 1991.

55. **Stenmark KR, McMurtry IF.** Vascular remodeling versus vasoconstriction in chronic hypoxic pulmonary hypertension: a time for reappraisal? *Circ Res* 97: 95–98, 2005.

56. **Stulc J, Stulcová B, Svihovec J.** Transport of calcium across the dually perfused placenta of the rat. *J Physiol* 420: 295–311, 1990.

57. **Talbert D, Sebire NJ.** The dynamic placenta: I. Hypothetical model of a placental mechanism matching local fetal blood flow to local intervillus oxygen delivery. *Med Hypotheses* 62: 511–519, 2004.

58. **Tissot van Patot MC, Bendrick-Peart J, Beckey VE, Serkova N, Zwerdlinger L.** Greater vascularity, lowered HIF-1/DNA binding, and elevated GSH as markers of adaptation to in vivo chronic hypoxia. *Am J Physiol Lung Cell Mol Physiol* 287: L525–L532, 2004.

59. **Vajner L, Vytásek R, Lachmannová V, Uhlík J, Konrádová V, Novotná J, Hampl V, Herget J.** Acute and chronic hypoxia as well as 7-day recovery from chronic hypoxia affects the distribution of pulmonary mast cells and their MMP-13 expression in rats. *Int J Exp Pathol* 87: 383–391, 2006.

60. **Walsh MP, Marshall JM.** The role of adenosine in the early respiratory and cardiovascular changes evoked by chronic hypoxia in the rat. *J Physiol* 575: 277–289, 2006.

61. **Zaidi SH, You XM, Ciura S, Husain M, Rabinovitch M.** Overexpression of the serine elastase inhibitor elafin protects transgenic mice from hypoxic pulmonary hypertension. *Circulation* 105: 516–521, 2002.

62. **Zamudio S.** The placenta at high altitude. *High Alt Med Biol* 4: 171–191, 2003.