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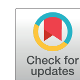
Pregestational diabetes increases fetoplacental vascular resistance in rats



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ABSTRACT

Introduction: Diabetes is a well-known risk factor in pregnancy. Because maternal diabetes involves oxidative stress that is also induced by chronic hypoxia and can alter vascular function, we sought to determine the effects of chronic maternal hyperglycemia on the fetoplacental vasculature in rats and to compare it with the effects of chronic hypoxia.
Methods: Diabetes was induced in female rats by a streptozotocin injection at a neonatal age. When these animals reached adulthood, their hyperglycemia was confirmed and they were inseminated. Half of them were exposed to hypoxia (10% O_2) for the last week before the delivery. One day before the expected date of delivery, one of their placentae was isolated and perfused.
Results: Fetoplacental vascular resistance was increased equally by experimental diabetes, chronic hypoxia, and their combination. Fetoplacental perfusion pressure-flow analysis suggested increased resistance in the small vessels in chronic hypoxia and in larger vessels in diabetes. Fetal plasma nitrotyrosine levels, measured as a marker of peroxynitrite (reaction product of superoxide and nitric oxide), mirrored the differences in fetoplacental resistance, suggesting a causative role. Fetoplacental vasoconstrictor reactivity to acute hypoxic stimuli was reduced similarly in all groups. Fasudil, a strong vasodilator agent, reduced fetoplacental vascular resistance similarly in all groups, suggesting that for the observed differences among the groups, the changes in vascular morphology were more important than variances in vascular tone.
Discussion: Maternal diabetes increases fetoplacental vascular resistance to a similar extent as chronic hypoxia. These stimuli are not additive. Changes in vascular tone are not responsible for these effects.
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1. Introduction

Chronic in utero hypoxia results in a sustained elevation of vascular resistance on the fetal side of the rat placenta [1]. The hypoxic state of the placenta could be a result of reduced availability of oxygen to the mother (e.g. maternal high altitude exposure or chronic respiratory disease of the mother) or could be a consequence of reduced uteroplacental blood flow (e.g. in preeclampsia or maternal heart failure). It is generally assumed that the hypoxic increase in fetoplacental vascular resistance leads to placental hypoperfusion and fetal undernutrition, and thus is a major factor in the pathogenesis of intrauterine growth restriction in situations like persistent maternal hypoxemia or chronic maternal vascular disorders [2]. The possible mechanisms responsible for the chronic hypoxic elevation of fetoplacental vascular resistance have not been elucidated, but based on many similarities between adult pulmonary circulation and fetoplacental vasculature [3] may involve oxidant injury of the vascular wall by oxygen radicals known to participate in the chronic hypoxic pulmonary hypertension [4–6].

Diabetes is one of the most common complications during pregnancy [7]. It is well known to affect fetal development [8–10]. Similarly to hypoxia, diabetes also induces oxidative stress. In particular, it causes overproduction of superoxide in the mitochondria of endothelial and myocardial cells (for review, see e.g. [11]). There is evidence that in diabetic pregnancies the fetus experiences chronic hypoxia [12,13].

The effects of maternal diabetes on fetoplacental vascular resistance have so far been little studied. The first aim of the

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present study, therefore, was to test the hypothesis that maternal diabetes causes an elevation of vascular resistance on the fetal side of the placenta. We also assumed that the relationships between diabetes, hypoxia, and vascular resistance in the fetal side of the placenta could be highlighted by combining the two factors (experimental diabetes and chronic maternal hypoxia) together.

2. Materials and methods

All procedures were in accordance with the European Guidelines on Laboratory Animal Care and were approved by the Animal Care Committee of the Second Faculty of Medicine, Charles University in Prague.

2.1. Experimental groups

All animals were fed ad libitum with free access to water and kept in regular light cycles of 12/12 h light/dark. Four groups of laboratory rats were used: one group had experimental diabetes (D, n = 9), another was exposed to chronic hypoxia (H, n = 9), the third had both diabetes and was exposed to hypoxia (DH, n = 9), and the fourth group were healthy control rats living in room air (C, n = 10). To induce experimental diabetes we used the model of beta cell regeneration after neonatal streptozotocin (STZ) injection [14]. Pregnant Wistar albino rats were obtained from commercial breeding colony (Velaz, Prague, Czech Republic). Pups were delivered spontaneously and nursed by their mothers. A single subcutaneous injection of STZ (Santa Cruz Biotechnology, Dallas, Texas, USA) in a dose of 100 mg/kg in 20 mM sodium citrate buffer solution (pH 4.5; Sigma-Aldrich, Munich, Germany) was administered on day 2 or 3 of postnatal life. Control animals received an equal volume of solvent. To confirm the diabetic state, the blood glucose concentration was monitored with a glucose analyzer (FreeStyleOptium, Abbott Diabetes Care Ltd., Maidenhead, UK) in the period between the 6th and 10th postnatal week. Blood was obtained by cutting off the very tip of the tail and squeezing it gently. Since, in line with reported values [15], the average glycemia in our control rats was 6.3 mmol/l (range 4.7–8.0) we included into the diabetic groups only animals with blood glucose concentration in excess of 8 mmol/l at least once during this period.

When adult (11th week of life), the diabetic and non-diabetic female rats were mated overnight with non-diabetic males. Then, when pregnant, they were randomly divided into two subgroups: the normoxic subgroup spent the whole gravidity in the atmospheric air, while the chronic hypoxic subgroup spent the last 7 days of pregnancy in a hypoxic normobaric chamber (10% O2) [16]. Experiments with perfused placenta were performed one day before the expected date of delivery (term = 21 days). We did not notice alterations in the length of gestation.

2.2. Perfused placenta preparation

We used the model of isolated, dually perfused rat placenta as previously reported [1,17]. Briefly, pregnant rats, anesthetized with sodium thiopental (Valeant, Prague, Czech Republic, 50 mg/kg i.p.), were placed into a bath of Ringer solution kept at 37 °C. After lower midline laparotomy the maternal side of selected placenta (uterine artery) was cannulated using a 24-gauge catheter and perfused at 1 ml/min with Krebs solution, whereas the uterine vein was ligated behind the perfused placenta and carefully cut to allow free drainage. The uterus was then opened on the antimesometrial side and the fetus was exposed. The umbilical artery of one of the placentae (chosen based on absence of visually apparent abnormalities and technical accessibility) was cannulated and perfused with the same perfusate as the maternal side from a common reservoir at a constant flow rate of 1 ml/min. At these baseline conditions, the common perfusate was gassed with a normoxic mixture of 21% O_2 and 5% CO_2 in nitrogen. Umbilical vein was punctured to permit an easy outflow of the perfusate. After rapidly establishing the perfusion, all fetuses were sacrificed with thiopental overdose.

2.3. Measurements

After the dual perfusion had been established, we measured fetoplacental perfusion pressure using a PowerLab data acquisition system (ADInstruments, Spechbach, Germany); the perfusion pressure at the maternal side was also monitored. After the preparation was stabilized and perfused at baseline conditions for at least 15 min, the perfusion pressure-flow relationship was determined by measuring fetoplacental perfusion pressure while the perfusion flow rate was continuously increased from 0 up to 2 ml/min during 120 s (P/Q ramp).

The flow rate was then returned to the baseline value of 1 ml/min and the acute hypoxic vasoconstrictor reactivity was measured by changing the gas mixture bubbling the perfusate to anoxic one (95% N_2 + 5% CO_2) until the fetoplacental perfusion pressure stabilized at a new level (20 min). After that, a Rho-kinase inhibitor fasudil (LC Laboratories, Woburn, Massachusetts, USA) was added to the perfusate (10 μM) in order to assess the vasoconstrictor component of the vascular resistance [17]. At the end of the perfusion, the mother was sacrificed by thiopental overdose. The perfused placenta, the other, non-perfused placentae from the same mother and all fetuses were weighed.

Blood samples were collected from the mothers' tails at the beginning of each experiment to measure hematocrit (by microcapillary tube centrifugation), glycemia, and plasma 3-nitrotyrosine. Plasma 3-nitrotyrosine was measured as an indicator of nitrating species, especially peroxynitrite [18], by inhibition ELISA (using antibodies prepared in our laboratory) as described previously [19]. Fetal blood was collected from the fetal body after decapitation and used to measure glycemia and plasma 3-nitrotyrosine.

2.4. Data analysis

The results were analyzed statistically using the Prism software version 7 (GraphPad Software, Inc., CA, USA). The groups were compared with two-way ANOVA (one factor normoxia vs. chronic hypoxia, second factor healthy vs. diabetes). The effects of acute hypoxic challenge and of fasudil were evaluated using two-way repeated measures ANOVA. In all cases, post hoc analysis was performed using Fisher's LSD test. $P < .05$ was considered significant. Linear regression was used to evaluate the P/Q ramp data. The results are presented as means ± SEM.

3. Results

Mother rats subjected to chronic hypoxia (H and DH groups) had lower body weight than normoxic groups, whereas experimental diabetes alone did not significantly affect maternal weight (Table 1). The body weight of the fetuses was not affected by either hypoxia alone or diabetes alone, but the combination of diabetes plus hypoxia resulted in significantly smaller fetuses (Table 1). The weights of the placentae did not differ among the groups, so the placental-fetal ratio was higher in the DH group than in the normoglycemic groups that did not differ one from another (Table 1). In all groups, the placental weight after the perfusion did not differ from the weight of placentae that were not perfused (Table 1), indicating that the perfusion protocol did not result in gross edema.

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