Production of NO and Oxidative Destruction of Proteins in the Placenta during Normal Pregnancy and Placental Insufficiency

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Study of NO generation and oxidative destruction of proteins showed intensification of these processes in the placenta during normal gestation. Activity of NO synthase and the concentration of nitrites in the placenta in women with placental insufficiency and spontaneous abortions were below the normal. Full-term pregnancy against the background of placental insufficiency was associated with a compensatory increase in NO production in the placenta. On the other hand, oxidative destruction of proteins increased in placental insufficiency irrespective of pregnancy outcome.

Key Words: *nitrogen oxide; oxidative destruction of proteins; placental insufficiency*

Placental insufficiency is an integral manifestation of various complications of pregnancy, sometimes leading to abortion [4,5]. Many problems associated with impairment of placental hemodynamics and fetomaternal circulation in this condition deserve further investigation.

An important role in the development of these processes is played by inadequate production of vasoactive substances, *e.g.* NO [8,11]. Impairment of NO production can involve, apart from alteration of the vascular tone, other dysregulatory effects, which is explained by extremely wide spectrum of its action (up to modulation of gene apparatus) [9]. NO modulates radical oxidative processes largely determining the conditions of intrauterine fetal growth [2].

Since NO production is limited by activity of NO synthase [10], the study of activity of this enzyme in the placenta can extend our notions on its role in the regulation of function and metabolism of this organ in normal and complicated pregnancy.

We studied NO production and oxidative destruction of proteins in the placenta during placental insufficiency.

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MATERIALS AND METHODS

Chorions and placentas of 22-28-year-old women (n=83) were examined. Eighteen of these women had medical abortions at 6-8 weeks (group 1), 20 had uncomplicated pregnancy ended in term delivery (group 2), 20 had complicated pregnancy eventuated in spontaneous abortion at 36-37 weeks (group 3), and in 25 patients pregnancy was complicated by placental insufficiency, but ended in term delivery (group 4). Placental insufficiency was diagnosed on the bases of hormone assay and measurements of specific placental isoenzymes (alkaline phosphatase and glutamate dehydrogenase) [3].

Chorionic and placental tissues collected immediately after medical abortion or delivery were washed from blood at 0-4°C and homogenized in 10% normal saline. Activity of NO synthase was evaluated by the increase in NO production from L-arginine in the presence of NADPH [6]. The amount of produced NO was evaluated by EPR spectroscopy of mononitrosyl complexes with bivalent iron and diethyldithiocarbamate, characterized by paramagnetic properties [12]. EPR signals were recorded on a Zeiss EP-9 radiospectrometer. Endogenous NO in the form of anion nitrite (NO_2^-) was measured using Griss reagent [7]. Oxi-

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dative destruction of proteins was evaluated by the level of carbonyl derivatives in the reaction with 2,4-dinitrophenylhydrasine [1].

The results were processed statistically using Statistica 5.1 software (StatSoft. Inc.). The results were significant at $p<0.05$.

RESULTS

In normal pregnancy activity of NO synthase and the content of NO_2^- in mature placenta are higher than in the chorion at early terms of gestation by 45 and 49%, respectively ($p<0.01$; Fig. 1, *a*). The increase in NO production by the end of pregnancy seemed to be due to the development of placental vascular network and the need of the organ adaptation to changing requirements of the fetus. Similar changes were observed in oxidative destruction of proteins: the content of carbonyl derivatives in the placenta on gestation weeks 39-40 (group 2) was 40% higher than that in the chorion at 6-8 weeks (group 1) ($p<0.01$).

A positive correlation between NO synthesis and the degree of oxidative modification of proteins ($r=0.77$ and 0.65; $p<0.05$, for the chorion and placenta, respectively) was found, which attests to interrelationship and mutual dependence between these processes.

In order to verify this hypothesis, we carried out *in vitro* experiments on chorion and placenta homogenates with addition of Nω-nitro-L-arginine (L-NNA), a classical inhibitor of NO synthase. L-NNA (1% solution) was added to the homogenates and the concentrations of NO_2^- and carbonyl derivatives were measured after 60 min. Homogenate aliquots containing normal saline instead of NO synthase inhibitor served as the control.

The decrease in nitrite concentration was paralleled by a decrease in oxidative destruction of proteins (Fig. 2), more pronounced in the chorion (by 29%, $p<0.05$) compared to mature placenta (by 18.4%, $p<0.05$). This regularity confirmed the important role of NO in the formation of free-radical status of the placental tissue, especially at the early terms of pregnancy. By the end of gestation the effect of NO production on free-radical oxidation in the placenta decreases.

Activity of NO synthase was 40% higher ($p<0.01$) and the level of NO_2^- 47% higher ($p<0.01$) in placentas developing under conditions of functional insufficiency in pregnancy eventuating in delivery in comparison with normal pregnancy (Fig. 1, *b*). In women with miscarriages activity of NO synthase and NO_2^- content in the placenta decreased by 39 and 46%, respectively, in comparison with these parameters in normal pregnancy ($p<0.01$). Decreased production of NO undoubtedly disturbs the balance of vasoactive components and leads to blood flow reduction, which was confirmed by Doppler measurements of fetal and maternal hemodynamics in these women. Enhanced NO production (we should say, unexpected) in placentas from group 4 women seemed to be compensatory and maintained the placental hemodynamics under conditions of complicated pregnancy, which helped to prolong pregnancy to a full term, though with a risk of miscarriage.

In contrast to opposite changes in NO production in the placenta (groups 3 and 4), modification of oxidative destruction of proteins was co-directed irrespective of pregnancy outcome. The content of carbonyl derivatives in placentas in group 3 and 4 was higher by 62% and 50% ($p<0.01$ both) compared to that in group 2.

The positive correlation between NO production and oxidative destruction of proteins ($r=0.73$, $p<0.05$)

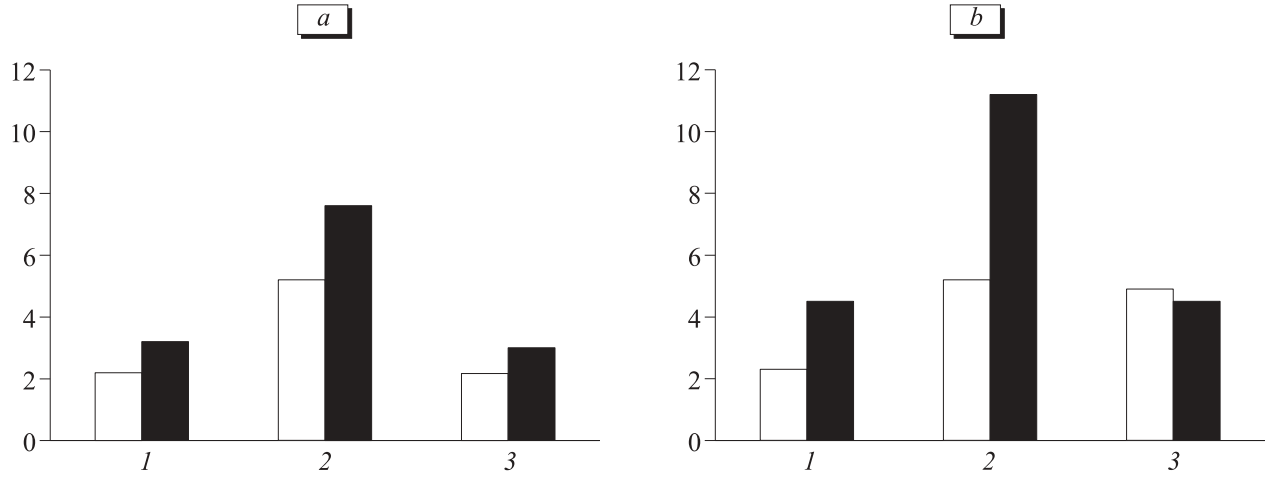

Fig. 1. Activity of NO synthase (nmol/g tissue) and content of NO_2^- (nmol/g tissue) and carbonyl derivatives (mmol/g) in the chorion and placenta in normal pregnancy (*a*) and placental insufficiency (*b*). *1*) NO synthase; *2*) NO; *3*) carbonyl derivatives. *a*) light bars: group 1; dark bars: group 2; *b*) light bars: group 3; dark bars: group 4.

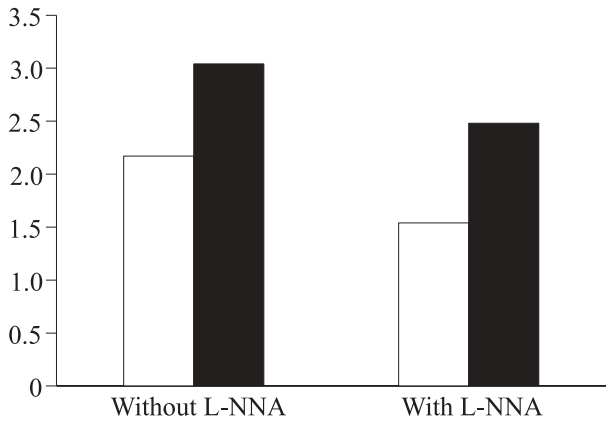

Fig. 2. Content of carbonyl derivatives (mmol/g) in the chorion and placenta in normal pregnancy after *in vitro* addition of Nω-nitro-arginine (L-NNA). Light bars: group 1; dark bars: group 2.

was also observed in full-term pregnancy with placental insufficiency. However, in case of abortion these parameters showed a trend to negative correlation (r= -0.42, p=0.05). These data attest to more intricate relationships between NO generation and free-radical oxidation of biosubstrates under conditions of complicated gestation, than could be expected proceeding from their physiological ratios.

Thus, placental insufficiency develops against the background of intensified oxidative destruction of proteins and changed NO production; the direction of these changes is essential for pregnancy outcome.

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