Granulocyte superoxide anion production and regulation by plasma factors in normal and preeclamptic pregnancy

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Abstract

Data on the respiratory burst activity of granulocytes from healthy and preeclamptic women have remained contradictory. To investigate the role of reactive oxygen species in the etiology of preeclampsia we measured superoxide anion generation by granulocytes from non-pregnant, healthy, and preeclamptic women. We also examined the reciprocal effects of heat-inactivated and non-inactivated plasma on superoxide production. Superoxide generation was measured by ferricytochrome-c reduction. Superoxide production induced by either phorbol-12.13-dibutirate or N-formyl-methionyl-leucyl-phenylalanine was significantly decreased in granulocytes from normal pregnant women compared with non-pregnant and preeclamptic women. The phorbol-12.13-dibutirate-induced superoxide generation by granulocytes from non-pregnant and preeclamptic women was significantly inhibited by plasma from healthy pregnant women. The N-formyl-methionyl-leucyl-phenylalanine-stimulated superoxide production by granulocytes from non-pregnant and preeclamptic women was suppressed only by non-inactivated plasma, not heat-inactivated plasma from healthy pregnant women. Plasma from preeclamptic women did not influence the phorbol-12.13-dibutirate- and N-formyl-methionyl-leucyl-phenylalanine-induced superoxide production by control granulocytes. The phorbol-12.13-dibutirate-induced superoxide generation by granulocytes from healthy pregnant women was significantly increased by the effect of plasma from non-pregnant and preeclamptic women, but when stimulating with N-formyl-methionyl-leucyl-phenylalanine only non-inactivated plasma caused the same enhancement. These data indicate that reduced superoxide generation in normal pregnancy may be caused by maternal immunosuppressive factors present in plasma. The failure to reduce superoxide production in preeclampsia may be partly responsible for the endothelial dysfunction characteristic of that condition.

Key words: preeclampsia; normal pregnancy; granulocytes; superoxide anion production; oxidative stress; plasma factors

1. Introduction

Preeclampsia is a pregnancy-specific multisystem disorder that develops in 3–10% of human pregnancies and is the leading cause of maternal and perinatal morbidity and mortality, especially in developed countries (Davey and MacGillivray, 1988). It is characterized by an abnormal vascular response to placentation, resulting in mild to severe maternal hypertension, proteinuria, enhanced platelet aggregation and activation of the blood coagulation system (Sibai et al., 2005). In addition to genetic factors maternal endothelial cell damage and dysfunction have been proposed to be implicated in the pathogenesis of the disease (Mellembakken et al., 2002). Although the exact mechanism is not yet known, an excessive maternal inflammatory response to pregnancy and the activation of granulocytes by placentally released circulatory factors have been suggested to contribute to endothelial damage in preeclampsia (Aly et al., 2004). Interaction of these factors with granulocytes may induce the production of superoxide anions ($O_2^{\cdot -}$) and related reactive oxygen species (ROS) (Hubel, 1999). Superoxide anions generated by activated granulocytes may initiate oxidative stress, lipid peroxidation and endothelial cell lysis (Gratacós, 2000).

Studies on $O_2^{\cdot -}$ production by granulocytes from normal and preeclamptic women have also provided conflicting results. The data published by Tsukimori et al. (1993) demonstrated that N-formyl-methionyl-leucyl-phenylalanine (FMLP)-stimulated $O_2^{\cdot -}$ generation by granulocytes was increased in women with preeclampsia compared with healthy pregnant women, and no significant difference was found between $O_2^{\cdot -}$ production by granulocytes from normal pregnant women and that by granulocytes from healthy non-pregnant women. Selvaraj et al. (1982) showed a higher granulocyte $O_2^{\cdot -}$ level in normal pregnancy compared with non-pregnant controls. Sacks et al. (1998) measured an increasing oxygen radical production of granulocytes in normal pregnancy and preeclampsia, but were unable to demonstrate a significant difference between these groups. In contrast, Crocker et al. (1999;

2000) reported that the FMLP-induced granulocyte $O_2^{\cdot -}$ generation was significantly reduced in healthy pregnant women compared with non-pregnant controls, and they found no significant difference between $O_2^{\cdot -}$ generation by granulocytes from patients with preeclampsia and granulocytes from healthy non-pregnant women. Crouch et al. (1995) and others have shown significantly lower granulocyte $O_2^{\cdot -}$ production in healthy pregnant women compared with non pregnant controls (Miller and Russell, 1986).

Several circulatory factors can influence $O_2^{\cdot -}$ production in normal and preeclamptic pregnancies (Redman and Sargent, 2003). There are suppressive effects in the maternal immune system in normal pregnancy; therefore, it is reasonable to suppose that immunosuppressive factors may also be present in the circulation of the mother (Viganò et al., 2007). For this reason the reduced $O_2^{\cdot -}$ generation in healthy pregnancy and normal $O_2^{\cdot -}$ production in women with preeclampsia may be due to the presence and absence of these factors in the plasma of normal and preeclamptic women, respectively. The purpose of our current study was to test this hypothesis. Therefore, we aimed to examine reciprocally whether plasma samples from healthy non-pregnant, normal and preeclamptic women could influence the phorbol-12,13-dibutirate- (PDBu) and FMLP-induced $O_2^{\cdot -}$ production by granulocytes from non-pregnant controls, and healthy and preeclamptic women. Furthermore, the $O_2^{\cdot -}$ production by granulocytes from healthy non-pregnant, normal and preeclamptic women was also measured using the above-mentioned stimulating agents.

2. Materials and methods

2.1. Study population

After informed consent and the approval of the Institutional Ethics Committee, peripheral blood was collected from 31 normal and 39 preeclamptic women in their third trimester of pregnancy. Blood samplings were performed in gestational weeks 26–38. A

group of 35 age-matched non-pregnant women served as controls in this study. Preeclampsia was defined as development of hypertension after the 20th week of pregnancy (≥ 140/90 mm Hg measured in two consecutive occasions 6 hours apart) and proteinuria of higher than 300 mg/day. The preeclamptic group included 9 mildly and 30 moderately ill patients with blood pressure of 140/90–149/99 mm Hg and 150/100–159/109 mm Hg, respectively (ACOG Committee on Obstetric Practice, 2002). All of the patients with preeclampsia were not on any medication, without a history of diabetes mellitus and with an absence of major medical disease or surgical intervention.

2.2 Separation of granulocytes from peripheral blood

Peripheral blood was collected in vacutainer test tubes containing EDTA (Becton-Dickinson, Cedex, France). Blood samples were layered on the top of a Ficoll solution (1.077 g/mL) and the supernatant containing the leukocytes was removed after sedimentation of erythrocytes at 1 *g* for 60 min at room temperature. The leukocyte-rich plasma was layered on top of a discontinuous Ficoll gradient (1.077 and 1.119 g/mL) and centrifuged at 350 *g* at 20°C for 30 min. Granulocytes sedimented at the interface of the Ficoll layers were collected and washed twice with Hanks' solution, pH 7.4 at 20°C (English and Andersen, 1974). Cell viability checked by the trypan blue exclusion test was found to be 98%. The purity of the granulocyte suspensions varied between 94 and 98% as revealed by microscopic examinations. Red blood cells were not removed by hypotonic lysis since the erythrocyte contamination in the granulocyte suspensions was negligible.

2.3. Measurement of superoxide anion production

Superoxide anion release was measured by superoxide dismutase-inhibitable (SOD, from bovine erythrocytes, 4200 U/mg protein) reduction of ferricytochrome-c (Babior et al.,

1975). Granulocytes ($3x10^5$) were incubated in Hanks' solution (pH 7.4) with phorbol-12,13-dibutyrate (PDBu) or n-formyl-methionyl-leucyl-phenylalanine (FMLP) at 37°C for 15 min. The total assay volume was 0.5 mL. The final concentrations of SOD, ferricytochrome-c, PDBu, and FMLP were 100 U/mL, 50 μmol/L, 100 nmol/L, and 1 μmol/L, respectively. The change in absorbance was measured spectrophotometrically at 550 nm with a double beam Shimadzu UV-160A spectrophotometer (Shimadzu Seisakusho Ltd., Kyoto, Japan) at room temperature. The amount of superoxide anion secreted into the medium was calculated on the basis of the molar extinction coefficient of reduced cytochrome-c $2.1x10^4$ $M^{-1}cm^{-1}$ (Pick and Keisari, 1981).

2.4. Effect of plasma samples on superoxide anion production by granulocytes from non-pregnant, healthy pregnant, and preeclamptic women

Plasma fractions were isolated from peripheral blood of healthy non-pregnant and normal pregnant women as well as patients with moderate preeclampsia by centrifugation at 800 *g* at 20°C for 10 min and then the plasma samples were divided into two parts. To check the suggested effect of the complement system on the superoxide production of granulocytes (Rossi, 1986) inactivated and non-inactivated plasma samples were used. Half of each fraction was heated to 56°C for 30 min, while the other portion was not inactivated. Subsequently, the individual plasma preparations were not pooled. Every experiment was performed with inactivated and non-inactivated plasma samples simultaneously. Granulocytes ($3x10^6$) from healthy non-pregnant women were incubated with plasma samples (1.5 ml) from normal and preeclamptic women. Granulocytes ($3x10^6$) from normal pregnant women were treated with plasma preparations (1.5 ml) from healthy non-pregnant and preeclamptic women. Granulocytes ($3x10^6$) from preeclamptic women were incubated with plasma fractions (1.5 ml) of non-pregnant and healthy pregnant women. In order to examine the

possibility of stimulation or suppression of $O_2^{\cdot -}$ production due to an immune reaction of the cells with foreign plasma factors, granulocytes ($3x10^6$) from non-pregnant, healthy pregnant, and preeclamptic women were also treated with autologous and heterologous plasma samples (1.5 ml) of non-pregnant, normal, and preeclamptic women respectively. Following incubation of the cells at 37°C for 1 h, granulocytes were washed with Hanks' solution and $O_2^{\cdot -}$ production was measured as described above.

2.5. Statistical analysis

The results are presented as means (±SD). The distribution of data, checked by the Kolmogorov–Smirnov test, was normal. Differences among the clinical parameters of the study population, superoxide anion production by granulocytes from non-pregnant controls, normal pregnant women, and preeclamptic women as well as $O_2^{\cdot -}$ generation by granulocytes from non-pregnant, healthy pregnant, and preeclamptic women treated with appropriate plasma samples were determined by one-way analysis of variance (ANOVA) using the Newman–Keuls post-hoc test. The data in Figs. 1-3 represent the mean values (±SD) obtained from independent experiments with granulocytes and plasma samples isolated from six individual women per group. Values of $p<0.05$ were considered to be statistically significant.

3. Results

The clinical parameters of the study population are presented in Table 1. There were significant differences in systolic and diastolic blood pressures ($p<0.001$), body mass index (BMI, $p<0.05$), gestational age at delivery ($p<0.01$), proteinuria at the time of blood sampling ($p<0.001$), and birth weight ($p<0.01$) between normal pregnant and preeclamptic women.

3.1. Superoxide anion production by granulocytes

Superoxide anion production by granulocytes from healthy non-pregnant, normal pregnant, and preeclamptic women in response to PDBu and FMLP stimulation is illustrated in our previous work (Lampé et al., 2008). As shown there, using the two stimulator agents (PDBu and FMLP), granulocytes from normal pregnant women demonstrated significantly decreased $O_2^{\cdot -}$ generation compared with non-pregnant and preeclamptic women. There was no significant difference in $O_2^{\cdot -}$ production by granulocytes from non-pregnant controls and that by granulocytes from patients with preeclampsia. Granulocytes from preeclamptic women released a significantly greater amount of $O_2^{\cdot -}$ compared with normal pregnant women.

3.2. Effects of plasma samples on superoxide anion production

The effects of plasma fractions from normal pregnant and preeclamptic women on $O_2^{\cdot -}$ production by granulocytes from healthy non-pregnant women are illustrated in Fig. 1A and 1B. As shown in Fig. 1A, both inactivated plasma (IP) and non-inactivated plasma (NIP) from healthy pregnant women significantly inhibited the PDBu-induced $O_2^{\cdot -}$ generation by granulocytes from non-pregnant controls compared with $O_2^{\cdot -}$ generation by the cells treated with autologous and heterologous IP and NIP from healthy non-pregnant women as well as preeclamptic women. There were no significant differences in PDBu-stimulated $O_2^{\cdot -}$ generation by granulocytes from non-pregnant controls after incubation of the cells with autologous and heterologous plasma from non-pregnant women as well as plasma from preeclamptic women. As depicted in Fig. 1B, the FMLP-stimulated $O_2^{\cdot -}$ production by granulocytes from non-pregnant controls was not influenced by IP from healthy pregnant and preeclamptic and heterologous non-pregnant women. However, incubation of the cells with NIP from healthy pregnant women resulted in a significant inhibition of $O_2^{\cdot -}$ generation

compared with $O_2^{\cdot -}$ production by granulocytes treated with autologous and heterologous NIP from non-pregnant and preeclamptic women.

The results of reciprocal plasma experiments with granulocytes from healthy pregnant women are demonstrated in Fig. 2. Both IP and NIP from healthy non-pregnant and preeclamptic women significantly increased the PDBu-induced $O_2^{\cdot -}$ generation by granulocytes from normal pregnant women compared with $O_2^{\cdot -}$ generation by granulocytes incubated with IP and NIP from autologous and heterologous healthy pregnant women (Fig. 2A). There were no significant differences in the PDBu-stimulated $O_2^{\cdot -}$ generation following incubation of the cells with autologous and heterologous healthy pregnant plasma. IP from healthy non-pregnant and preeclamptic women as well as heterologous healthy pregnant women did not influence the FMLP-stimulated $O_2^{\cdot -}$ production by granulocytes from normal pregnant women (Fig. 2B). In contrast, incubation of the cells with NIP from non-pregnant and preeclamptic women resulted in a significant increase in $O_2^{\cdot -}$ generation compared with $O_2^{\cdot -}$ production by granulocytes treated with autologous and heterologous NIP from healthy pregnant women.

The effects of plasma fractions from non-pregnant controls and healthy pregnant women on $O_2^{\cdot -}$ production by granulocytes from preeclamptic women are presented in Fig. 3. Treatment of the cells with IP and NIP from healthy pregnant women caused a significant decrease in the PDBu-induced $O_2^{\cdot -}$ generation by granulocytes from patients with preeclampsia compared with $O_2^{\cdot -}$ release by granulocytes incubated with autologous and heterologous IP and NIP from preeclamptic women (Fig. 3A). There were no significant differences in PDBu-stimulated $O_2^{\cdot -}$ production following incubation of the cells with autologous and heterologous preeclamptic plasma as well as non-pregnant plasma. Exposure of granulocytes from preeclamptic women to IP from non-pregnant, and autologous and heterologous preeclamptic women did not cause significant changes in the FMLP-stimulated

$O_2^{\cdot -}$ generation (Fig. 3B). However, treatment of the cells with NIP from healthy pregnant women resulted in a significant reduction in $O_2^{\cdot -}$ generation compared with $O_2^{\cdot -}$ production by granulocytes incubated with autologous and heterologous NIP from preeclamptic women.

4. Discussion

Data on the respiratory burst activity of granulocytes from healthy and preeclamptic women have remained contradictory. Although numerous studies have reported that there is no significant difference between the stimulated $O_2^{\cdot -}$ production by granulocytes from non-pregnant controls and that by granulocytes from healthy pregnant subjects (Tsukimori et al., 1993), pregnancy-related depression of granulocyte ROS generation has also been reported by several researchers (Crouch et al., 1995; Miller and Russell, 1986; Kindzelskii et al., 2002, 2004). In order to further investigate the involvement of granulocyte-derived free radicals in the endothelial injury in preeclampsia, we measured $O_2^{\cdot -}$ production by granulocytes from non-pregnant controls, healthy women, and preeclamptic women. Our results suggest that one of the most important granulocyte functions, the generation of ROS, is suppressed in physiological pregnancy, which is consistent with the findings of previous studies demonstrating depression of granulocyte ROS generation in healthy pregnant women. This reduction may be explained by a general suppression of cell-mediated immunity that has been demonstrated in pregnancy (Terness et al., 2007). The attenuation of $O_2^{\cdot -}$ production may be required to protect fetal and maternal cells from the granulocyte-mediated oxidative damage in normal pregnancy. A similar decrease in ROS generation was not detected in patients with preeclampsia, which may be due to disturbances in the maternal immune system and may be partly responsible for endothelial injury in preeclampsia.

Epidemiological studies have supported the fact that the incidence of bacterial and viral infections is higher among pregnant women (Persellin and Thoi, 1979; Jamieson et al.,

2006). As granulocyte-derived ROS play a pivotal role in the destruction of pathogenic micro-organisms (Witko-Sarsat et al., 2000), our results and previous findings indicating decreased $O_2^{\cdot -}$ generation in physiological pregnancy are in agreement with these epidemiological data. In addition, sera from pregnant women have been demonstrated to inhibit bacterial killing and phagocytosis by control granulocytes (Persellin and Leibfracth, 1978). Similarly, a suppressive effect of both IP and NIP from healthy pregnant women on the PDBu-induced $O_2^{\cdot -}$ generation by granulocytes from non-pregnant controls and patients with preeclampsia was observed in our study. In contrast, depression of the FMLP-stimulated $O_2^{\cdot -}$ production was not detected, when granulocytes from non-pregnant and preeclamptic women were incubated with IP from healthy pregnant women. This suggests that the plasma factor responsible for the suppression of FMLP-induced respiratory burst may be heat-sensitive and was therefore degraded during heating. To exclude this possibility, control and preeclamptic granulocytes were also treated with NIP from normal pregnant women. The results of these experiments indicated that NIP from healthy pregnant women was able to decrease the FMLP-stimulated $O_2^{\cdot -}$ generation by granulocytes from non-pregnant and preeclamptic women. The inhibition was probably not associated with the effect of foreign plasma factors since there was no significant difference in the PDBu- and FMLP-stimulated $O_2^{\cdot -}$ production by granulocytes from normal and preeclamptic women after incubation of the cells with autologous and heterologous plasma samples. Our investigations also indicated that preeclamptic plasma did not increase PDBu- and FMLP-induced $O_2^{\cdot -}$ generation by control granulocytes.

To show that the depression of $O_2^{\cdot -}$ production was mediated by the healthy pregnant plasma, granulocytes from normal pregnant women were incubated with plasma from non-pregnant and preeclamptic women. The results of these experiments demonstrated that the decreased ROS generation by healthy pregnant granulocytes could be increased by treatment of the cells with non-pregnant and preeclamptic plasma. In the case of PDBu stimulation, both

IP and NIP samples were able to enhance the $O_2^{\cdot -}$ production by healthy pregnant granulocytes. The FMLP-stimulated $O_2^{\cdot -}$ production by normal pregnant granulocytes exposed to non-autologous and heterologous NIP was also improvable by treatment of the cells with NIP from non-pregnant and preeclamptic women. In response to FMLP, $O_2^{\cdot -}$ production by granulocytes from normal pregnant women treated with autologous and heterologous IP samples showed a similar $O_2^{\cdot -}$ release to granulocytes from healthy pregnant women treated with IP from non-pregnant and preeclamptic women. This finding also suggests that the healthy pregnant plasma factor responsible for the inhibition of FMLP-induced respiratory burst was inactivated during heating, and was therefore not able to suppress $O_2^{\cdot -}$ generation.

The mechanisms by which a plasma factor of healthy pregnant women can inhibit the $O_2^{\cdot -}$ production by granulocytes from non-pregnant and preeclamptic women are not yet known. Our results suggest that the factor in question may affect the signal transduction pathways resulting in the activation and translocation of PKC from the cytosol to the plasma membrane. This has been reported to induce a sequence of events leading to the translocation of the cytosolic components of NADPH oxidase to the cell membrane and subsequently the assembly of the active $O_2^{\cdot -}$ generating enzyme complex (Nauseef et al., 1991). The agonists used in our experiments can activate PKC in different pathways. Following binding to formyl peptide receptors (FPRs), FMLP has been shown to initiate the hydrolysis of phosphatidylinositol-4,5-bisphosphate, resulting in the production of inositol-1,4,5-trisphosphate and diacylglycerol (DAG), which activates PKC (Panaro et al., 2006). PDBu mimics the action of DAG, bypasses receptor-mediated signaling events and directly activates PKC (Goel et al., 2007). We suppose that the NIP factor might interact with FPRs, leading to a decrease in their ligand-binding capacity; therefore, the receptors might be less efficient in transducing signals required for the induction of $O_2^{\cdot -}$ production. With heat treatment, the plasma factor in question appears to undergo denaturation that abolishes its ability to

influence receptor-mediated signaling. However, further studies are required to specify this factor.

In conclusion, $O_2^{\cdot -}$ production by granulocytes is decreased in normal pregnancy, which may be due to a defense mechanism to protect the maternal and fetal cells against granulocyte-mediated oxidative damage. The deficient $O_2^{\cdot -}$ generation in normal pregnancy may be caused by maternal immunosuppressive circulatory factors. The failure to reduce $O_2^{\cdot -}$ production in preeclamptic women may be partly responsible for endothelial injury. However, further studies are required to confirm this conclusion.

Acknowledgments

This work was supported by the Hungarian National Research Fund (Grant Nr.: T 048964) and the Ministry of Education of Hungary (Contract No. NKFP1-00003/2005). The authors thank Mrs. Mariann Kovács for her excellent technical assistance.

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Figure Legends

Figure 1.

Effect of plasma samples on PDBu- (A) and FMLP-stimulated (B) superoxide anion production by granulocytes from non-pregnant controls. Granulocytes were incubated with inactivated (IP) and non-inactivated (NIP) plasma from healthy and preeclamptic women as well as with autologous and heterologous non-pregnant plasma. Mean values (±SD) obtained from independent experiments with granulocytes and plasma samples isolated from six individual women/group are demonstrated. Significant differences are indicated as follows: ***$p < 0.001$ autologous IP versus healthy pregnant IP; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ autologous NIP versus NIP from healthy pregnant women.

Figure 2.

Effect of plasma samples on PDBu- (A) and FMLP-induced (B) superoxide anion production by granulocytes from healthy pregnant women. Granulocytes were incubated with inactivated (IP) and non-inactivated (NIP) plasma samples from non-pregnant controls and preeclamptic women as well as with autologous and heterologous plasma from normal pregnant subjects. Mean values (±SD) obtained from independent experiments with granulocytes and plasma samples isolated from six individual women/group are presented. Significant differences are indicated as follows: ***$p < 0.01$ autologous IP versus healthy non-pregnant IP and preeclamptic IP; $^{\#\#\#}p < 0.001$ autologous NIP versus NIP from healthy non-pregnant and preeclamptic women.

Figure 3.

Effect of plasma samples on PDBu- (A) and FMLP-stimulated (B) superoxide anion production by granulocytes from preeclamptic women. Granulocytes were incubated with inactivated (IP) and non-inactivated (NIP) plasma samples from non-pregnant controls and healthy pregnant subjects as well as with autologous and heterologous plasma from preeclamptic women. Mean values (±SD) obtained from independent experiments with granulocytes and plasma samples isolated from six individual women/group are shown. Significant differences are indicated as follows: *$p < 0.05$, ***$p < 0.001$ autologous IP versus healthy pregnant IP; $^{###}p < 0.001$ autologous NIP versus NIP from normal pregnant women.

Table 1.

Clinical characteristics of non-pregnant (NP), healthy pregnant (HP), and preeclamptic pregnant (PE) women

Clinical data	NP women (n=35)	HP women (n=31)	PE women (n=39)	p value
Age (years)	31.6 ± 2.8	31.1 ± 4.3	31.3 ± 3.9	>0.05
Gestational age at blood-sampling (weeks)	NA	32.1 ± 3.3	32.4 ± 4.9	>0.05
Gestational age at delivery (weeks)	NA	40.0 ± 1.4	37.4 ± 2.3	<0.05*
Pre-pregnancy BMI[b] (kg/m^2)	NA	24.3 ± 1.4	24.6 ± 1.6	>0.05
BMI at blood sampling (kg/m^2)	25.2 ± 1.4	27.4 ± 4.4	30.8 ± 4.2	<0.05*
Parity [a]	NA	0 (0–2)	0 (0–1)	>0.05
Gravidity [a]	NA	2 (1–3)	2 (1–3)	>0.05
Systolic blood pressure at blood sampling (mm Hg)	124 ± 4.7	121 ± 5.8	153 ± 7.8	<0.05*
Diastolic blood pressure at blood sampling (mm Hg)	80 ± 4.6	79 ± 6.5	104 ± 4.1	<0.05*
Neonatal weight (g)	NA	3533.1 ± 538	3008 ± 736	<0.05*
Proteinuria at blood Sampling, urine dipstick [a]	0 (0–0)	0 (0–0)	3 (1–3+)	<0.05*

Mean values ± SD are presented. [a]Values are expressed as median (range). NA: not applicable. Significant differences are indicated as follows: *p<0.05 healthy pregnant versus preeclamptic pregnant women, [b]BMI: body mass index

Figure 1.

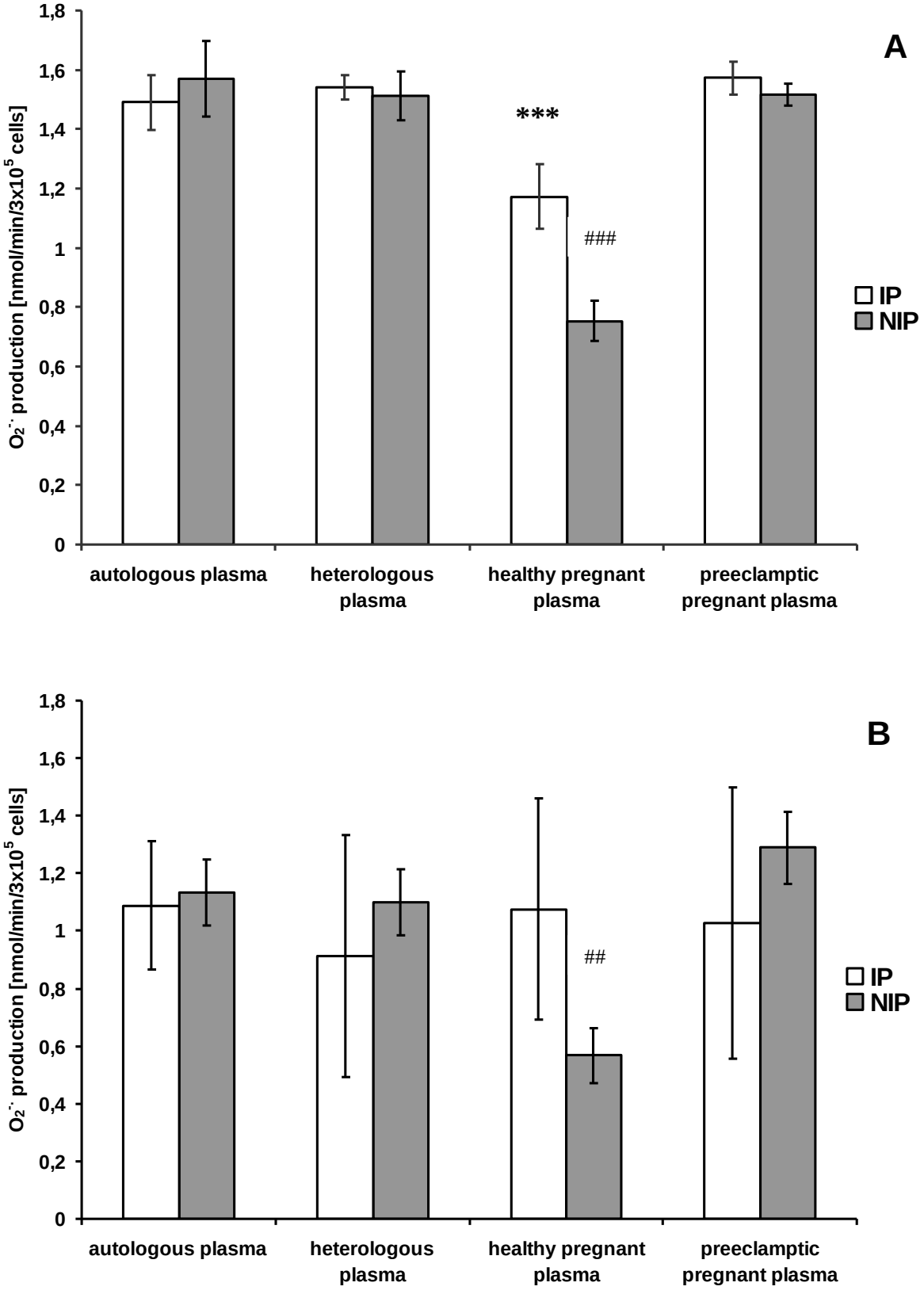

A
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
$O_2^{\cdot-}$ production [nmol/min/3x10^5 cells]
***
###
IP
NIP
autologous plasma
heterologous plasma
healthy pregnant plasma
preeclamptic pregnant plasma
B
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
$O_2^{\cdot-}$ production [nmol/min/3x10^5 cells]
##
IP
NIP
autologous plasma
heterologous plasma
healthy pregnant plasma
preeclamptic pregnant plasma

Figure 2.

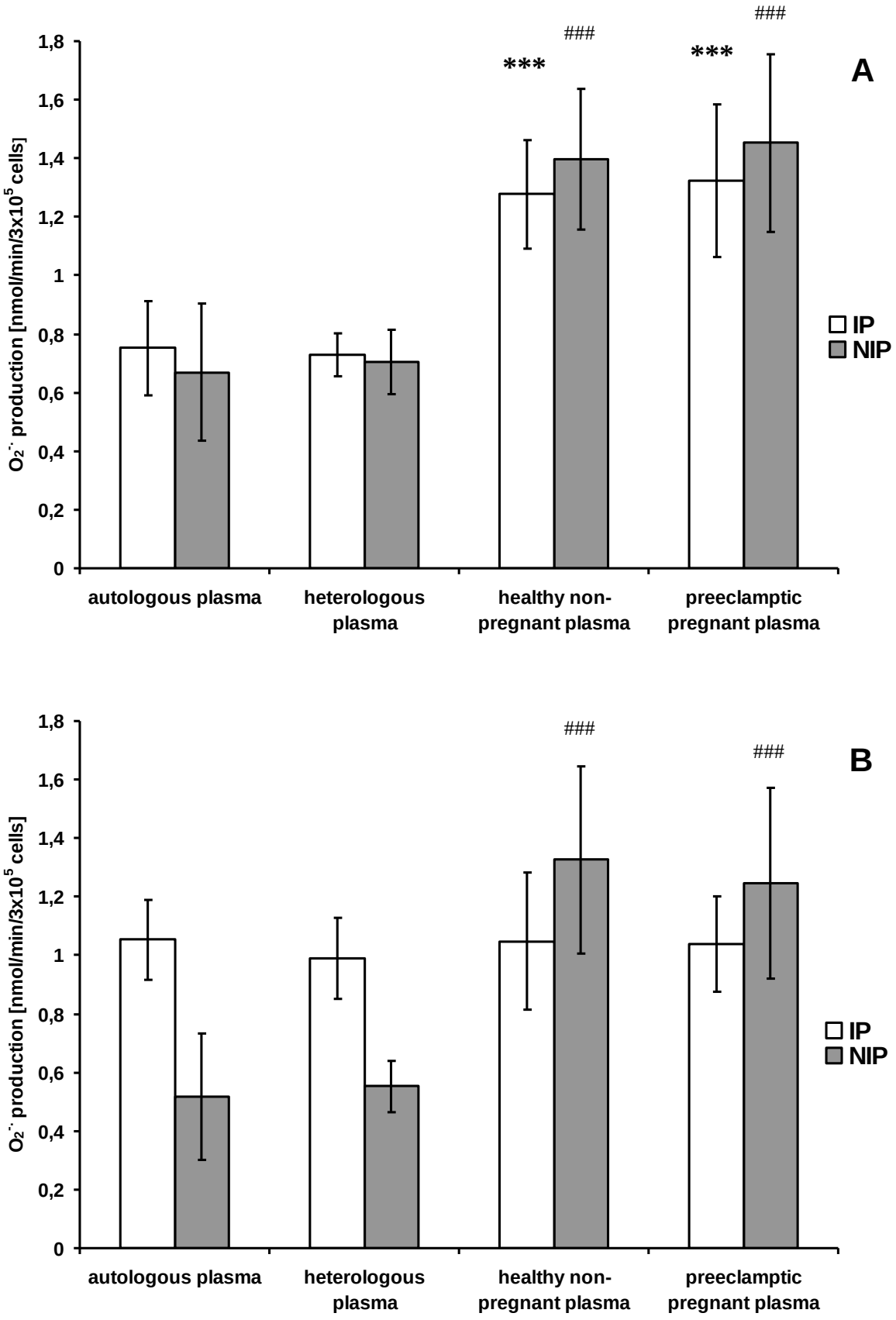
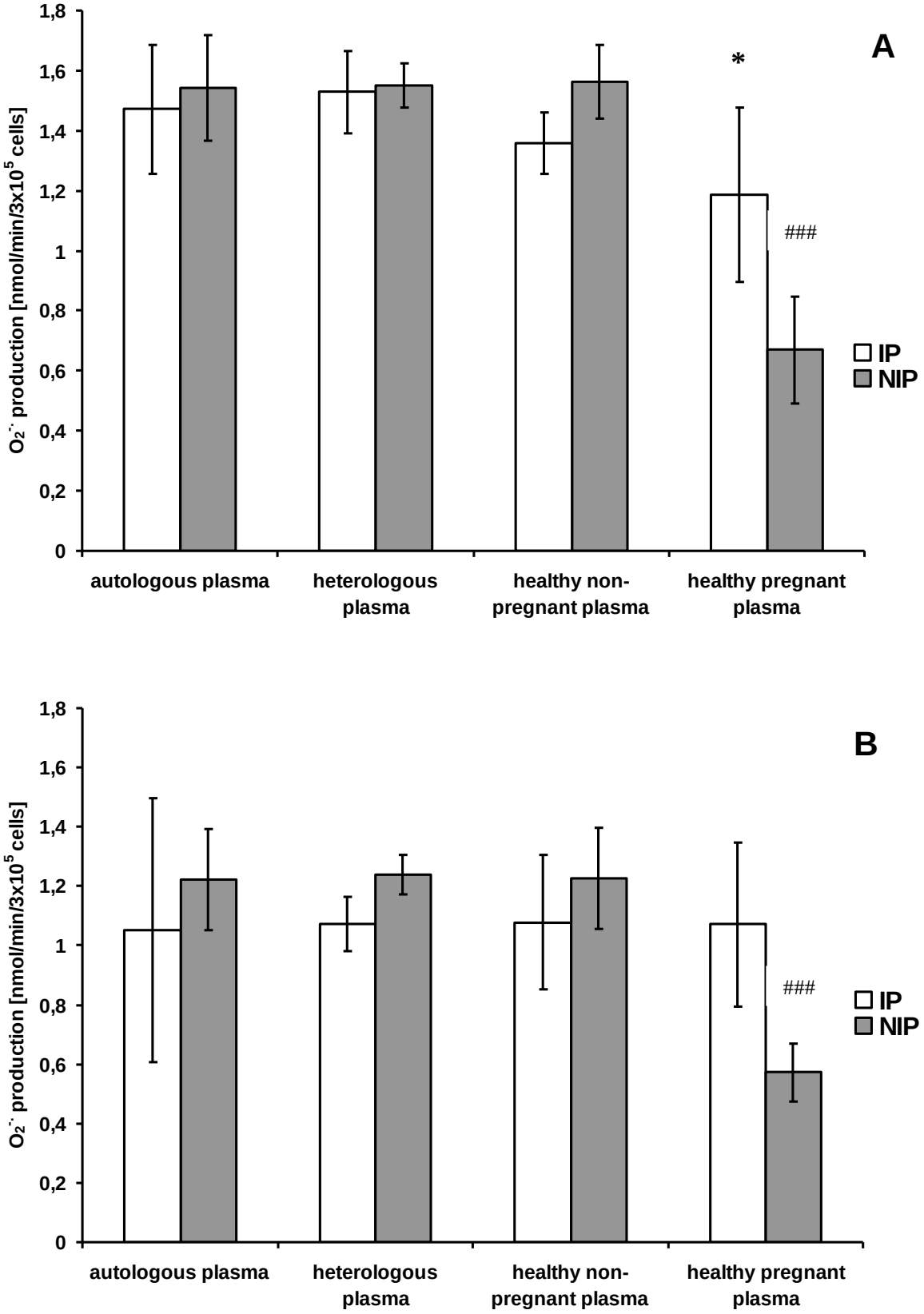

A
B
IP
NIP
$O_2^{\cdot-}$ production [nmol/min/3x10^5 cells]
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
***
###
autologous plasma
heterologous plasma
healthy non-pregnant plasma
preeclamptic pregnant plasma

Figure 3.


A
B
$O_2^{\cdot-}$ production [nmol/min/3x10^5 cells]
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
*
###
IP
NIP
autologous plasma
heterologous plasma
healthy non-pregnant plasma
healthy pregnant plasma