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The expression and activity of monoamine oxidase A, but not of the serotonin transporter, is decreased in human placenta from pre-eclamptic pregnancies

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Abstract

Serotonin (5-HT) plays a pivotal role in pregnancy and a hyperserotonomic condition has been documented in pre-eclampsia. We have attempted to elucidate the possible participation of 5-HT as an aetiological factor in pre-eclampsia, by estimating the activity and expression of the 5-HT transporter (SERT) and monoamine oxidase A (MAO-A) in human placenta from full term normal (NG) and severe pre-eclamptic (PES) pregnancies. Uptake of 5-[1,2-^{3}H] hydroxytryptamine binoxalate (specific radioactivity, 30.4 Ci/mmol) was determined in placental brush border vesicles by a rapid filtration technique. 5-HT metabolism in placental homogenate was measured using a HPLC-ECD system. Expression of SERT and MAO-A was determined by Western blot, using specific antibodies against the human SERT and MAO-A in placental tissues obtained from NG and PES. Our results, showed no significant difference in 5-HT uptake between both groups. However, 5-HT metabolism was significantly lower in placental homogenates from PES than in NG placentas, with the pathological preparations showing no MAO-A activity against 5-HT during the first 60 min of incubation (87% and 5% of metabolism of 5-HT initially added, NG and PES respectively). Western blot analysis showed a similar expression of SERT in BBMV from NG and PES. However, unlike for normal pregnancies, the expression of MAO-A in placental homogenates from PES was found to be very low, or almost negligible. These findings confirm our previous results and suggest that the higher plasma free 5-HT levels observed in severe pre-eclampsia could be mainly due to a reduction in placental MAO-A expression and activity and are not limited by the expression and uptake of 5-HT into the placental tissue.

Keywords: Pre-eclampsia; Serotonin; Placenta; MAO-A

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Introduction

Pre-eclampsia is considered to be one of the most significant health problems in human pregnancy, complicating 6–8% of all gestations over 20 weeks [1]. This disease is one of the leading causes of fetal growth retardation and mortality, premature birth and maternal death [2]. Despite continuous advances in research, pre-eclampsia remains a major challenge in both understanding its pathophysiology and management. Today, it is unanimously viewed as a multisystem disorder with vascular dysfunction at its centre [3,4].

Although the exact cause of pre-eclampsia remains to be elucidated, evidence shows that serotonin (5-Hydroxytryptamine, 5-HT) may play a substantial role in the pathogenesis of this disease and a hyperserotonomic condition has been documented in pre-eclampsia [5,6].

In our earlier studies we have shown that 5-HT causes intense vasoconstriction in isolated human placental vessels [7], and that threshold concentrations of 5-HT increase sensitivity to other vasoconstrictors [8,9]. Also, we recently reported an increase in plasma 5-HT concentration in pre-eclampsia as compared with normotensive pregnant women [10], however, we were unable to detect a difference in the platelet 5-HT concentration between women with established severe pre-eclampsia and their matched normotensive pregnant controls. Finally, we attempted to elucidate the possible participation of 5-HT as an aetiological factor in pre-eclampsia, by estimating the activity of the 5-HT transporter (SERT) and monoamine oxidase A (MAO-A) in human placenta from full term normal (NG) and severe pre-eclamptic pregnancies (PES) [11]. Our results showed that there was no significant difference in 5-HT uptake (V_{max}= 6.25 ± 0.76 and 5.82 ± 0.74 pmoles(mg protein × 30 sec)$^{-1}$, NG and PES, respectively and K_m= 59.2 ± 5.84 and 53.90 ± 3.90 nM, NG and PES, respectively) by the placental brush border membranes of the syncytiotrophoblast between NG and PES subjects. However, 5-HT metabolism was significantly lower in placental homogenates from PES than in NG placentas, with the pathological preparations showing no MAO-A activity against 5-HT during the first 60 min of incubation.

In the present study we characterized the expression of human placenta 5-HT transporter and monoamine oxidase A from full term NG and PES pregnancies. We assesed the possible contribution of placenta into increased circulating 5-HT concentration in the mother, which may give account for some of the pathophysiological features of pre-eclampsia.

Materials and methods

The subjects consisted in 7 healthy women with normal pregnancies, and 7 women with severe pre-eclampsia. Pre-eclampsia was defined as the 2 following symptoms: a sustained rise of blood pressure to more than 150/100 mmHg and proteinuria of more than 5 g/day. The diagnosis of pre-eclampsia was made during prenatal care by a hospital committee and obstetricians specialized in high-risk pregnancies. Essential or secondary hypertension and chronic renal diseases were excluded.

Transport experiments in syncytiotrophoblast plasma membranes

Maternal-facing brush border vesicles (BBMV) were prepared from human term placentas from NG and PES according to Balkovetz et al. [12]. The purity of preparations was assessed

by measuring the enrichment of the brush border enzyme alkaline phosphatase [13]. The brush-border membranes used in this study were enriched in alkaline phosphatase activity 20- to 25-fold compared to the homogenate obtained from placenta of NG and PES.

Uptake of 5-[1,2-^{3}H] hydroxytryptamine binoxalate (^{3}H-5-HT, 30 seconds, 51 and 155 nM, 4μCi/ml) in BBM vesicles was determined by a rapid filtration technique as described by Ramamoorthy et al. [14].

Serotonin metabolism by homogenate of human term placenta

Placental homogenate was obtained from the central part of the maternal placental surface and this preparation was used to characterize monoamine oxidase activity with HPLC with electrochemical detector [11]. When indicated, samples were preincubated with clorgyline 1 μM, a high specific MAO-A inhibitor.

Western blotting

Expression of SERT and MAO-A was determined in BBMV or in placental homogenate, respectively. Briefly, 50 μg of protein were submitted to 10% sodium dodecyl sulphate-poliacrilamide gels electrophoresis (SDS-PAGE), then transfered electrophoretically by semi-dry blot on PVDF membranes. The membranes were blocked with 5% fat-free milk powder in PBS for 2 hours. Immunodetection was performed with either anti human SERT (1:1000) or anti human MAO-A (1:1000), overnight at 4°C, followed by incubation with peroxidase-labelled anti-rabbit antibody. The bound antibodies were visualized by enhanced chemiluminescence according to the instructions of the manufacturer (Amersham). Documentation was performed by exposure to X-ray film (Fuji, Japan). As negative controls either irrelevant rabbit immunoglobulins were applied or the incubation step with the primary antibodies was omitted.

Drugs and statistics

^{3}H-5-HT, specific radioactivity 30.4 Ci/mmol, was purchased from Du Pont-New England Nuclear. Serotonin and imipramine were obtained from Sigma. Clorgyline was purchased from RBI, USA. All other chemicals were of analytical grade.

Anti human SERT antibody was obtained from (Alpha Diagnostics Inc, USA). Anti human MAO-A antibody was a gift of Dr. A.Parini (INSERM; Toulouse, France).

Results are given as mean ± SEM. Statistical differences were determined by Student's t test. p values < 0.05 were considered statistically significant.

Results

Patient characteristics

The clinical characteristics of pregnant women are summarized in Table 1. There were no significant differences between NG and PES subjects in either gestational age ($p>0.29$) or maternal age ($p>0.65$). PES patients demonstrated significantly higher systolic pressure ($p<0.0001$), diastolic pressure ($p<0.0001$), proteinuria ($p<0.001$) and lower birth weight

Table 1
Clinical characteristics of the normal pregnant women and women with severe pre-eclampsia

	Normal pregnant	Severe pre-eclampsia
N	7	7
Maternal age years	24.1 ± 1.2	23.4 ± 1.0
Nulliparity	5 of 7	6 of 7
Gestational age (weeks)	38.5 ± 0.2	38.1 ± 0.3
Diastolic pressure (mmHg)	67.3 ± 1.0	114.3 ± 2.0**
Systolic pressure (mmHg)	112.1 ± 1.7	161.1 ± 2.9**
Proteinuria (g/day)	—	6.78 ± 0.6**
Platelets (×1000/μl)	223 ± 3.6	107 ± 5.9**
Gestational weight (g)	3551 ± 72.7	1823 ± 256**

The results are expressed as mean ± SEM. The symbol ** indicates $p < 0.01$ statistically significant differences from normal pregnant women.

($p<0.0001$). Platelet count was significantly lower ($p<0.0001$) in women with PES than in NG group.

SERT activity and expression in BBMV from NG and PES

Placental BBMV obtained from NG and PES was studied incubating 30 seconds with ^{3}H-5HT (55 and 155 nM, 4 μCi/ml). As shown in Fig. 1A, the uptake of [^{3}H]-5-HT in BBMV was similar between NG and PES groups. Western blot studies showed a single band for the SERT (62 Kda approx.). The expression of SERT did not differ significantly between NG and PES samples (Fig. 1B). Platelet membrane vesicles was used as a positive SERT control (Data not shown).

MAO-A activity and expression in homogenate of human placenta

When placental homogenate of NG was incubated with 5-HT, more than 84% of the substrate added initially was metabolized within 60 minutes of incubation (Fig. 2A, open bars). This effect was inhibited by clorgyline 1 μM, a specific MAO-A blocker (Fig. 2A, dashed bars). No metabolism of 5-HT by placental homogenates of PES was detected during 60 min of incubation (Fig. 2A, closed bars). Immunoblots using an anti-human-MAO-A antibody showed a single band for MAO-A (57 Kda approx., Fig. 2B). The expression of MAO-A in placental homogenate from PES was very low, or even almost negligible.

Discussion

In this study we present novel data showing no significant difference in SERT expression in the placental BBMV between NG and PES subjects, suggesting that in both groups of pregnants the SERT participate similarly in the clearance of 5-HT from the maternal circulation. On the contrary, the expression of the MAO-A was very low, or almost undetectable in the homogenate of the placenta from PES in comparison with homogenate of normotensive placenta, where a significant higher activity and expression of this enzyme was found.

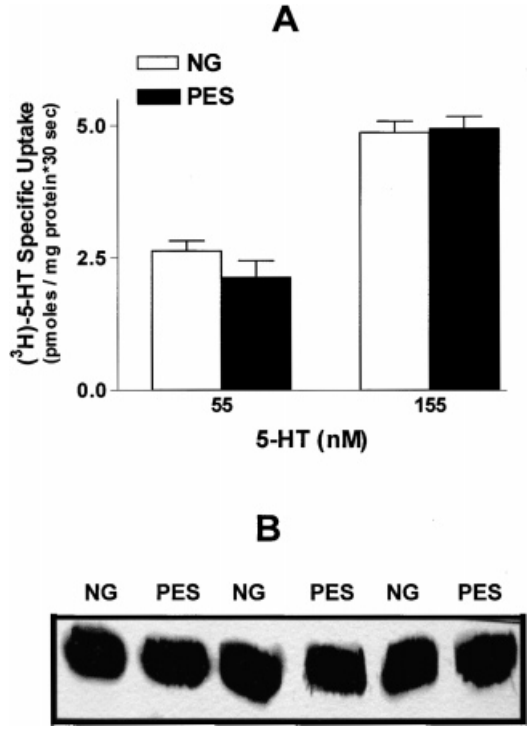

Fig. 1. **Activity (A) and expression (B) of the SERT in human placental vesicles from NG and PES pregnancies.** (A) [^{3}H]- 5HT specific uptake in BBMV from NG and PES. There were no significant differences in the uptake values at any concentration studied (B) Immunoblots of SERT in BBMV was performed after SDS-PAGE 10% minigels (50 μg of protein per lane). No significant differences in the SERT expression were detected between NG and PES samples. Results are representative of seven different experiments.

Previous studies have found a small decrease in placental monoamine oxidase activity in pre-eclampsia compared to controls, whereas others have failed to detect any significant difference [15]. Recently, Gujrati et al. [6] demonstrated a significant reduction of placental monoamine oxidase activity in pre-eclampsia, and this decrease showed a correlation with severity of the disease. Another important finding of these authors was to demonstrate the presence of enzymatic activity of monoamine oxidase in nuclei and cytoplasm of the syncytiotrophoblast cells in pre-eclampsia; in contrast to normal placenta that showed high activity in the cytoplasm without any activity in the nucleus of syncytiotrophoblastic cells.

The pathophysiological implicances of the decreased MAO-A activity and the increasing placental 5-HT are speculative. Marley et al. [16], have proposed that the placental accumulation of 5-HT would be followed by diffusion of the monoamine to the uterine endometrium, causing constriction of the blood vessels with consequent decrease in the placental blood supply. On the other hand, there is evidence, from animal studies, that the placenta is capable of transferring 5-HT from the maternal side to the fetal side at least in the early stages of pregnancy [17], where 5-HT has been proposed as an important factor in fetal development [18]. Due to cytotoxic properties of higher 5-HT concentration in culture cells, a possible

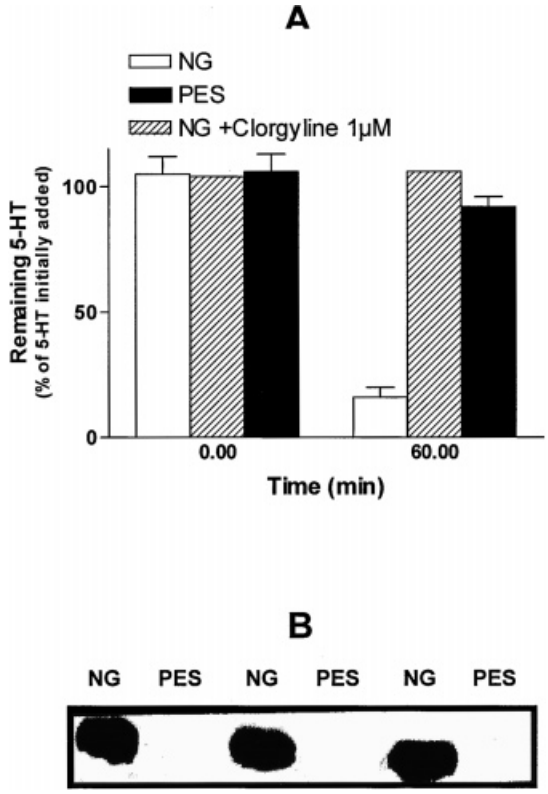

Fig. 2. **Activity (A) and Expression (B) of MAO-A in human placental homogenate from NG and PES:** (A) Placental homogenates of PES were unable to metabolize 5-HT during 60 min of incubation. Mean ± SEM of percentages of 5-HT added initially. (B) Western Blot of MAO-A in human placental homogenate from NG and PES were performed after SDS-PAGE 10% minigels. The expression of MAO-A in placental homogenates from PES was lower than in NG samples. Results are representative of seven different samples.

link could be established between increased placental 5-HT and the IUGR (Intra-Uterine-Growth-Restriction) detected in pre-eclamptic pregnancies.

Gujrati et al. [6], also proposed an additional source of 5-HT in placenta, that includes the possible transfer of 5-HT from the fetus to the mother. The fetus produces 5-HT, which is released into fetoplacental circulation, and is partially metabolized by placental monoamine oxidase and the rest goes to the maternal circulation [6,19,20]. In severe pre-eclampsia, where placental MAO-A is highly reduced, a subsequent rise of 5-HT in the placenta and finally in mother plasma could be found. This may lead to intravascular aggregation of platelets in situ, resulting in release of platelet content. This local release of 5-HT would lead to vasoconstriction, resulting in ischemia and microinfarcts in chorionic villi, characteristic of severe pre-eclampsia.

Recent results from our laboratory and others indicated that although 5-HT platelet transport is increased in pre-eclamptic subjects, 5-HT plasma concentration in this syndrome is significantly higher than that measured in the plasma of normotensive pregnant women [10],

suggesting that in pre-eclamptic pregnants the possible transfer of 5-HT from the placental tissue or the decreased placental metabolism could induce a raise in the mother 5-HT plasma levels.We speculate that a major physiological role of intracellular monoamine oxidase is to keep cytosolic 5-HT concentration very low, to enable the monoamine carriers to produce a net inward transport of 5-HT, and thereby, to act as the first step in the termination of action of extracellular 5-HT. This offers an effective mechanism to maintain this vasoactive monoamine at very low level in the intervillous space. These processes are physiologically important to the placental function and fetal development because 5-HT, if not efficiently cleaned from the intervillous space, would cause vasoconstriction of uterine arteries and reduce the uteroplacental circulation. This will produce deleterious effects on the developing fetus because exchange of nutrients and metabolic waste products between the maternal and fetal circulations will be compromised due to the decreased blood flow through the intervillous space [14].

5-HT is among the most potent vasoconstrictors of the umbilicoplacental circulatory bed [20], where it is thought to be involved in the humoral control of vascular tone [21]. Some authors have proposed that only 5-HT, bradykinin, and oxytocin induce contractile responses at sufficiently low physiologic concentrations, to be involved in the control of the vascular tone in umbilicoplacental circulation [21,23]. Additionally, 5-HT has been implicated in causing increased vascular permeability, in eliciting renal ischemia and subsequent renal cortical necrosis, and in enhancing vascular sensitivity to such vasocontrictive agents as histamine and $PGF_2\alpha$ [8,9]. Depending on the integrity of the vascular endothelium, interaction with 5-HT results in vasodilation or vasoconstriction. If there is some intact endovascular endothelium, 5-HT may interact with $5HT_1$ receptors located on endothelial cells, resulting indirectly in local vasodilation by stimulating the local release of prostacyclin and nitric oxide [24,25]. When endothelium is seriously dysfunctional, as it is assumed to be in severe early-onset pre-eclampsia [26], the $5HT_1$ receptor-mediated response may be diminished. 5-HT is believed to react with $5HT_2$ receptors on smooth muscle cells and platelets, resulting in direct vasoconstriction, amplifying the effects of other vasoconstrictors and augmenting the effects of platelet aggregation and thrombus formation.

This study describes the possible cellular processes responsible for the hyperserotonomic condition associated with pre-eclampsia. The results presented here convincingly show no differences in the expression of the placental SERT between NG and PES. However, there are marked differences in the expression of MAO-A. The expression is almost undetectable in pre-eclamptic placentas. These data suggest that pre-eclampsia is associated with increased 5-HT in the placenta and possibly in the intervillous space mainly due to the decreased activity of MAO-A.

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