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Transport and Metabolism of Serotonin in the Human Placenta from Normal and Severely Pre-Eclamptic Pregnancies

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Key Words
Serotonin · Pre-eclampsia · Placenta · Serotonin uptake · Monoamine oxidase

Abstract
We have attempted to elucidate the possible participation of serotonin as an etiological factor in pre-eclampsia. The transport of serotonin into vesicles from the maternal-facing brush border membrane was measured, as well as the metabolism induced by monoamine oxidase (MAO) in placental homogenate obtained from normal-term and severely pre-eclamptic placentas. Kinetic analysis of serotonin uptake by the placental brush border membrane of the syncytiotrophoblast between normally pregnant and severely pre-eclamptic subjects showed no significant difference (similar Vmax and Km values). However, the metabolism of serotonin was significantly higher in placental homogenate from normal pregnancies than in placentas from severely pre-eclamptic pregnancies. These findings suggest that the higher plasma-free serotonin levels observed in severe pre-eclampsia are mainly due to a reduction in MAO-A activity and not limited by the rate of serotonin uptake into the cells.

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Introduction

Pre-eclampsia is one of the more common complications associated with pregnancy and a major cause of maternal and perinatal mortality. It is described as a maternal syndrome characterized by hypertension and proteinuria. It has been proposed that these and other clinical signs are secondary to a placental pathology, probably placental ischaemia resulting from uteroplacental arterial insufficiency [1, 2].

The multi-system nature of the maternal syndrome could not be explained until generalized maternal endothelial cell dysfunction was suggested as the underlying problem [3, 4]. It is presumed that this is caused either directly or indirectly by one or more circulating factors derived from the placenta. The placenta can contribute to pre-eclampsia through numerous mechanisms, one of which is to increase circulating serotonin, thereby augmenting vasoconstriction and platelet aggregation that impede normal blood flow [5]. The important role of increased serotonin levels in pre-eclampsia has been documented [5, 6]. In our earlier studies, we have shown that serotonin causes intense vasoconstriction in isolated human placental vessels [7], and that threshold concentrations of serotonin increase sensitivity to other vasocon-

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strictors [8, 9]. Furthermore, the presence of a specific 5-HT_2 receptor has also been demonstrated in the umbilical and placental vessels [10, 11]. We recently also reported an increase in plasma serotonin concentration in pre-eclampsia as compared with normotensive pregnant women. However, we were unable to detect a difference in platelet serotonin concentration between women with established proteinuric pre-eclampsia and their matched normotensive pregnant controls [12].

The syncytiotrophoblast of the human placenta expresses a Na^+- and Cl^--dependent serotonin transporter in the maternal-facing brush border membrane [13]. It has been speculated that this transporter is involved in the clearance of the vasoactive monoamine from the intervillous space to ensure optimal blood circulation in the uteroplacental bed. The functional significance of the placental serotonin transporter depends primarily on the fate of serotonin once it enters the trophoblast after being transported across the brush border membrane; it can either be degraded by monoamine oxidase (MAO), or transported into the fetal or maternal circulation. The human placenta contains high MAO-A activity [14], which has a higher affinity for serotonin and is inhibited by low concentrations of clorgyline [15]. Since the placental MAO is responsible for serotonin metabolism within the placenta, the activity of the serotonin transporter in the placental brush border membrane also plays an important role in the ability of the placenta to metabolize serotonin. The purpose of our study, therefore, was to clarify the dynamics of serotonin metabolism in placentae from normal and severely pre-eclamptic pregnancies.

Materials and Methods

The present study was conducted in pregnant women admitted to or attending the prenatal clinic at the Guillermo Grant Benavente Hospital, Concepción. This study was approved by the Institutional Ethical Committee and informed consent was taken from each patient before inclusion in the study.

We studied 12 women with normal pregnancy and 9 women with severe pre-eclampsia. Severe pre-eclampsia was diagnosed when any of the following criteria was present: blood pressure ≥160 mm Hg systolic or ≥110 mm Hg diastolic measured on two occasions at least 6 h apart with the patient at rest, or proteinuria ≥1 g in a 24-hour urine collection or ≥3 on a dipstick from two or more random clean-catch samples at least 4 h apart [16]. The diagnosis of pre-eclampsia was made during prenatal care by the hospital committee and obstetricians specializing in high-risk pregnancies. Essential or secondary hypertension and chronic and renal diseases were excluded.

The normal patients had no medical problems and remained normotensive throughout gestation without proteinuria. No subject was known to have chronic hypertension or renal or metabolic disease.

Preparation and Characterization of Syncytiotrophoblast Plasma Membranes

Maternal-facing brush border vesicles were prepared from human term placentae from normal and severely pre-eclamptic pregnancies within 15 min after delivery and placed in ice-cold NaCl (0.9%). All procedures were carried out at 4°C. Brush border plasma membranes (BBM) were obtained according to the method described by Balkovetz et al. [17]. The purity of preparations was assessed by measuring the enrichment of the brush border enzyme alkaline phosphatase [18]. There was no significant difference between BBM vesicles from normotensive women and BBM vesicles from women with severe pre-eclampsia with regard to alkaline phosphatase enrichment ($p > 0.50$).

Transport Experiments in BBM

Uptake of 5-[1,2-^{3}H] hydroxytryptamine binoxalate ([^{3}H]-5-HT; specific radioactivity 30.4 Ci/mmol) in BBM vesicles was determined by a rapid filtration technique as described by Ramamoorthy et al. [19] and characterized in terms of Michaelis-Menten kinetics.

Metabolism of Serotonin by Human Term Placental Homogenate

Placental homogenate was obtained from the central part of the maternal placental surface from normal and severely pre-eclamptic pregnancies, and this preparation was used to characterize MAO activity. Placental tissue (100 g) was washed three times with 5 vol of ice-cold NaCl (0.9%) and then homogenized in a Waring Blendor with 2.5 vol of ice-cold mannitol, buffer (300 m*M* mannitol, 10 m*M* Hepes-Tris, pH 7.4). A 2.0-ml sample was taken for protein analysis and proteinase inhibitors were added (1 mg/ml leupeptin, 1 mg/ml aprotinin, 0.1 mg/ml bacitracin and 0.1 mg/ml phenylmethanesulphonyl fluoride). The homogenate was then treated with a loose-fitting Teflon/glass homogenizer and centrifuged at 2,300 *g* for 10 min. The supernatant was collected and used to study serotonin metabolism. For MAO-A activity assays, 900 µl of supernatant were preincubated for 15 min at 37°C. In some cases, the samples were preincubated with 1 µ*M* clorgyline. The reactions were started by adding 100 µl of serotonin (5 m*M*) for an indicated time and were stopped by adding 0.2 ml of $HClO_4$ (3.4 *M*). Then the samples were centrifuged for 10 min at 4,500 *g*. The supernatant was used for serotonin remnant determination. Serotonin measurements were made as previously described [12] by using a high-performance liquid chromatography system. The reproducibility of the method was 0.1 ng serotonin.

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.

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

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Table 1. Clinical characteristics of women with normal pregnancy and women with severe pre-eclampsia

	Normal pregnancy (n = 12)	Severe pre-eclampsia (n = 9)
Maternal age, years	24.7 ± 1.0	23.9 ± 1.4
Nulliparity	3/12	6/9**
Gestational age, weeks	38.4 ± 0.8	37.9 ± 0.4
Diastolic pressure, mm Hg	69.8 ± 1.6	107.8 ± 3.2**
Systolic pressure, mm Hg	110.4 ± 2.6	159.8 ± 3.3**
Proteinuria, g/day	ND	7.0 ± 1.5**
Platelets, × 1,000/μl	218 ± 1.2	112 ± 9.0**
Gestational weight, g	3,457 ± 91.1	1,712 ± 380.1**

The results are expressed as mean ± SEM. ** $p < 0.01$, statistically significantly different from normally pregnant women. ND = Not detected.

Table 2. Kinetic constants of [^{3}H]-5-HT uptake in placental BBM vesicles of normally pregnant women and women with severe pre-eclampsia

	Normal pregnancy	Severe pre-eclampsia	p
Vmax, pmol/mg protein × 30 s	6.25 ± 0.76	5.82 ± 0.74	0.85
Km, n*M*	59.2 ± 5.84	53.9 ± 3.9	0.49

No significant differences in Km and Vmax values between groups. Values are mean ± SEM.

Results

Patient Characteristics

The various clinical characteristics of the pregnant women participating in this study are summarized in table 1. There were no significant differences between the normal and pre-eclamptic subjects in either gestational age ($p > 0.59$) or maternal age ($p > 0.63$). Patients with severe pre-eclampsia demonstrated significantly higher systolic pressure ($p < 0.01$), diastolic pressure ($p < 0.01$), proteinuria ($p < 0.01$) and lower birth weight ($p < 0.01$) and platelet count ($p < 0.01$).

[^{3}H]-5-HT Uptake by BBM Vesicles

The uptake rate of [^{3}H]-5-HT (51 n*M*) in human placental BBM vesicles obtained from placentae of normally pregnant women and women with severe pre-eclampsia was found to be linear, at least up to 45 s, and therefore a 30-second incubation was employed to measure the initial uptake rates to be used in the kinetic analysis. Consequently, [^{3}H]-5-HT-specific uptake by placental BBM vesicles obtained from normal and severely pre-eclamptic women was studied incubating for 30 s over a serotonin concentration range of 1–200 n*M*. The calculated apparent Km and Vmax values for [^{3}H]-5-HT uptake did not differ significantly ($p > 0.1$) among normally pregnant women and women with severe pre-eclampsia (fig. 1). Non-linear regression analysis of the data demonstrated that a single saturable transport system is present, since the data were best fitted by an equation for a single rectangular hyperbola. The calculated apparent Km and Vmax values from the two groups of subjects are summarized in table 2.

Metabolism of Serotonin by Human Placental Homogenate

Placental homogenate preparations from normal and severely pre-eclamptic pregnancies were used to characterize MAO activity which was determined by measuring the amount of serotonin remnant in the placental homogenate after 60 min of incubation with serotonin (0.5 m*M*). When the placental homogenate of normal pregnancies was incubated with serotonin, more than 85% of the substrate was metabolized within 60 min. The metabolism of serotonin was almost completely inhibited by 1 μ*M* clorgyline, indicating that this substrate was metabolized specifically by MAO-A. The metabolism of serotonin by placental homogenates of severely pre-eclamptic pregnancies was similarly analysed, and the data are shown in figure 2. In these preparations, more than 94% of serotonin remained in the homogenate after 60 min of incubation, showing no MAO activity.

Discussion

Pre-eclampsia only occurs during pregnancy and is believed to require a placental-derived factor for its initiation. It has long been proposed that the placental component of pre-eclampsia is mediated by reduced placental perfusion resulting in the production of circulating factors that alter endothelial cell function [2, 3]. Several studies have shown that the serotonin concentration in placenta and plasma is increased in pre-eclampsia [12, 20, 21]. It has been suggested that the placental accumulation of serotonin would be followed by diffusion of the mono-

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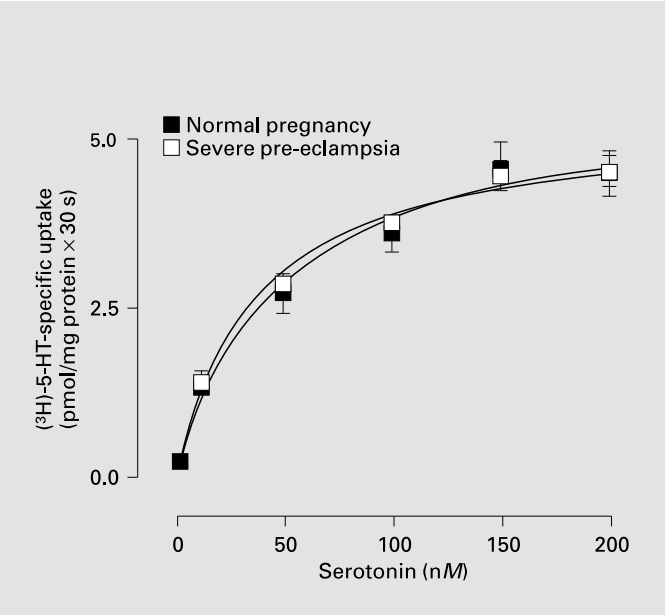

Fig. 1. [^{3}H]-5-HT-specific uptake and kinetic constants in human placental BBM vesicles from normal (n = 12) and severely pre-eclamptic (n = 9) pregnancies. Transport assays were performed as described in Methods.

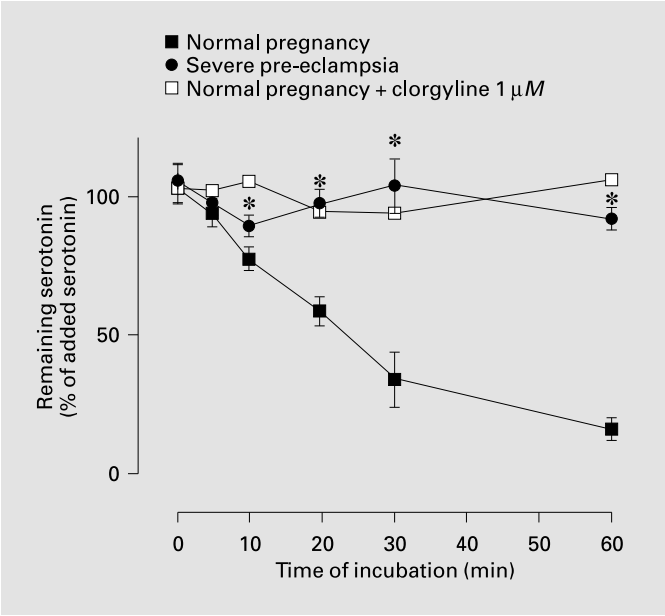

Fig. 2. Time course of serotonin metabolism by placental homogenate from normal (n = 12) and pre-eclamptic (n = 9) pregnancies. Values are significantly different from the respective control normal value. Mean ± SEM percentages of serotonin added initially.

amine into the uterine endometrium causing constriction of the blood vessels with a subsequent decrease in placental blood supply [22]. However, the mechanisms leading to the elevation of placental serotonin levels are still not known.

The study of the involvement of serotonin in pre-eclampsia was prompted by the fact that a serotonin-induced placental vasculature spasm was tenfold greater than that seen with norepinephrine [6]. Other experimental studies have shown that pharmacologic concentrations of serotonin administered intravascularly produced vasospasm, hypertension and oliguria, a syndrome similar to pre-eclampsia [23]. Recent results from our and other laboratories [12] indicate that although serotonin platelet transport is increased in pre-eclampsia, the serotonin plasma concentration in this syndrome is significantly higher than that measured in the plasma of normally pregnant women, suggesting that in pre-eclamptic pregnancy other factor(s) may contribute to increased plasma serotonin levels. Possible reasons for the higher levels of plasma-free serotonin include a decrease in the uptake of serotonin by the placenta or a decrease in the metabolic conversion of serotonin to its metabolite 5-hydroxyindolacetate by the placental MAO-A. Also, a decrease in the placental transporter activity would increase the levels of serotonin in the intervillous space which might contribute to the pathogenesis of pre-eclampsia.

In the present study, we demonstrated that there was no significant difference in serotonin uptake by the placental BBM of the syncytiotrophoblast between normally pregnant and severely pre-eclamptic subjects. These results suggest that in both pregnancy groups the serotonin transporter participates in a similar way in the clearance of serotonin from the maternal circulation.

Since there is no known mechanism to store the serotonin entering the syncytiotrophoblast from the maternal blood via the BBM serotonin transporter, this monoamine is either degraded inside the cell, or transported into the fetal circulation or to adjacent uterine endometrial areas. There is evidence that a major factor in the regulation of serotonin levels in the body is its metabolism by MAO [6]. Substrate and inhibitor specificity studies reveal that the placental MAO activity consists primarily of the form A that has a high affinity for serotonin [24]. Serotonin-synthesizing enzymes have not yet been reported in the placenta, therefore an impaired metabolism of serotonin may be a possible explanation for the elevation of placental serotonin levels in pre-eclampsia [25]. Our re-

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sults show that the serotonin metabolism was significantly higher in palcentae from normal pregnancies than in placentae from severely pre-eclamptic pregnancies. This indicates that the higher plasma-free serotonin levels observed in severe pre-eclampsia are due in in part to a reduction in MAO-A activity and not limited by the rate of serotonin uptake into the cells. Our study also demonstrates that normal placental cells apparently metabolize serotonin almost exclusively by MAO-A, as revealed by the use of clorgyline, a specific MAO-A blocker [26]. The low activity of MAO-A in pre-eclampsia allows the serotonin that is transported across the placenta to pass into the syncytiotrophoblast without being degraded and to be available for transfer into the fetal circulation. Some studies have found a small decrease in placental MAO activity in pre-eclampsia compared to controls whereas others have failed to observe any significant difference [27]. Further, Gujrati et al. [6] observed a significant reduction in placental MAO activity in pre-eclampsia, and this decrease showed a correlation with disease severity.

A major physiological role of intracellular MAO-A is to keep a low cytosolic serotonin concentration and to enable the monoamine carriers to produce a net inward transport of serotonin. This is an effective mechanism to maintain the serotonin at a very low level in the intervillous space. These processes are physiologically important to the placental function and fetal development because if serotonin is not efficiently cleared from the intervillous space, this would cause vasoconstriction of uterine arteries and reduce the uteroplacental circulation. Thus, the fetus would have alterations in its development because exchange of nutrients and metabolic waste products between the maternal and fetal circulations would be compromised due to the decreased blood flow through the intervillous space [28].

In this study, we demonstrated that the impairment of the placental MAO-A activity in pre-eclampsia appears to be an important factor leading to increased serotonin levels which induce intense vasoconstriction resulting in a compromise of the feto-placental circulation. This alteration may result in damage to vascular endothelial cells, thereby further promoting activation and aggregation of blood platelets, and presumably enhancing the pathology of pre-eclampsia.

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