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Short communication

Reduced expression of $Na^{(+)}/H^{(+)}$ exchanger isoform 3 (NHE-3) in preeclamptic placentas

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ABSTRACT

Although the etiology of preeclampsia is unknown, accumulated evidence suggests that the expression of a variety of syncytiotrophoblast transporters is reduced or abnormal. Here, we have examined the expression of NHE-3 in preeclamptic placentas. We found that NHE-3 expression significantly decreased and its labeling was almost undetectable in the cytosol of syncytiotrophoblast cells.

Even though the inductor mechanisms of NHE-3 decrease are not clear yet, we speculated that alterations in TNF-α and aldosterone levels observed in preeclampsia might be downregulating NHE-3 expression. Further studies are needed to define whether these alterations play a direct role either in the pathogenesis or in the adaptative response of preeclampsia.

1. Introduction

Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria. Although its etiology is unknown, accumulated evidence suggests that the expression of a variety of syncytiotrophoblast transporters is reduced or abnormal [1–5].

Some of the best documented alterations involve changes in the handling of sodium ion both on the systemic and on the cellular level. It is well known that a transmembrane sodium transport disorder causes an increase in intracellular sodium concentration which plays a critical role in the pathogenesis of essential hypertension [6]. Thus, the study of cell sodium handling in preeclampsia is increasingly gaining importance. There is broad agreement that the components of the renin–angiotensin–aldosterone pathway are markedly reduced in women with preeclampsia [7,8]. However other changes, especially those involving cell sodium are less consistent.

Many studies support an increase in peripheral cell sodium concentration suggesting a defect in the (NA, K) ATPase activity in preeclamptic placentas while other evidence indicates increased circulating concentrations of a sodium pump inhibitor [4,5].

On the other hand, Marino and co-workers have recently reported a diminished expression of the three subunits of the epithelial sodium channel (ENaC) in the membranes of preeclamptic placentas in comparison with the normal ones [3].

Regarding the study of $Na^{(+)}/H^{(+)}$ exchangers (NHEs) in the setting of preeclampsia, little is known. The NHEs are a widely expressed family of proteins that mediate electroneutral exchange of one extracellular Na^+ for one intracellular H^+ and subserve various functions including the maintenance of pHi, transepithelial Na^+ transport, cell volume homeostasis and cell proliferation [9,10]. These functions are all compromised in pregnancies complicated with preeclampsia. From the nine NHEs members identified to date, at least three isoforms (NHE-1, NHE-2 and NHE-3) have been found in normal human placenta [11]. All three isoforms are expressed in both apical and basal membranes, however data as to the predominant location of each isoform varies between different studies [9,11,12]. Interestingly, NHE-3 expression was also found in the cytoplasm [9,12] accordingly to its recycling capacity [13].

There has been one published study carried out on placentas taken from either normotensive or preeclamptic pregnancies. Activity was not measured but the expression of NHE-1 protein was found to be decreased in preeclamptic placentas [14]. In contrast, other study found increased NHE activity in the erythrocytes from preeclamptic women [15].

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Table 1
Clinical characteristics of severe preeclamptic and normotensive women. Values are mean ± SD. Severe preeclampsia was defined as systolic blood pressure ≥160 mmHg and/or diastolic pressure ≥110 mmHg, with proteinuria ≥0.3 g/day or 2 pluses on urine dipstick after the 20th week of gestation in a previously normotensive patient.

	Normotensive pregnant women	Severe preeclamptic pregnant women
Number of pregnant women	10	10
Parity		
Primiparous	4	5
Multiparous	6	5
Maternal age, yr	22.3 ± 1.1	23.4 ± 1.5
Gestational age, wk	38.8 ± 1.2	36.5 ± 0.9
Blood pressure, mmHg		
Systolic	110 ± 4.1*	160.0 ± 4.5*
Diastolic	64 ± 2.3**	113.0 ± 1.9**
Proteinuria	Negative	+++
Body mass index (BM), kg/m^2	25 ± 3	24 ± 4
Birth weight, g	3160 ± 210	2880 ± 240
Fetal sex		
Male	5	6
Female	5	4
β-hCG (IU/mL)	11.93 ± 2.33	31.68 ± 7.42*
TNF-α (pg/mL)	9.6 ± 1.3	15.3 ± 0.4*

*$P < 0.01$. **$P < 0.01$.

In this work, we focused on the isoform 3 of NHE (NHE-3) which differs from the others in that it recycles between the plasma membrane and intracellular compartments [13]. Therefore, NHE-3 expression and localization in the syncytiotrophoblast cells should be finely regulated by a variety of stimuli, both acutely and chronically, and any alterations might lead to a pathological condition.

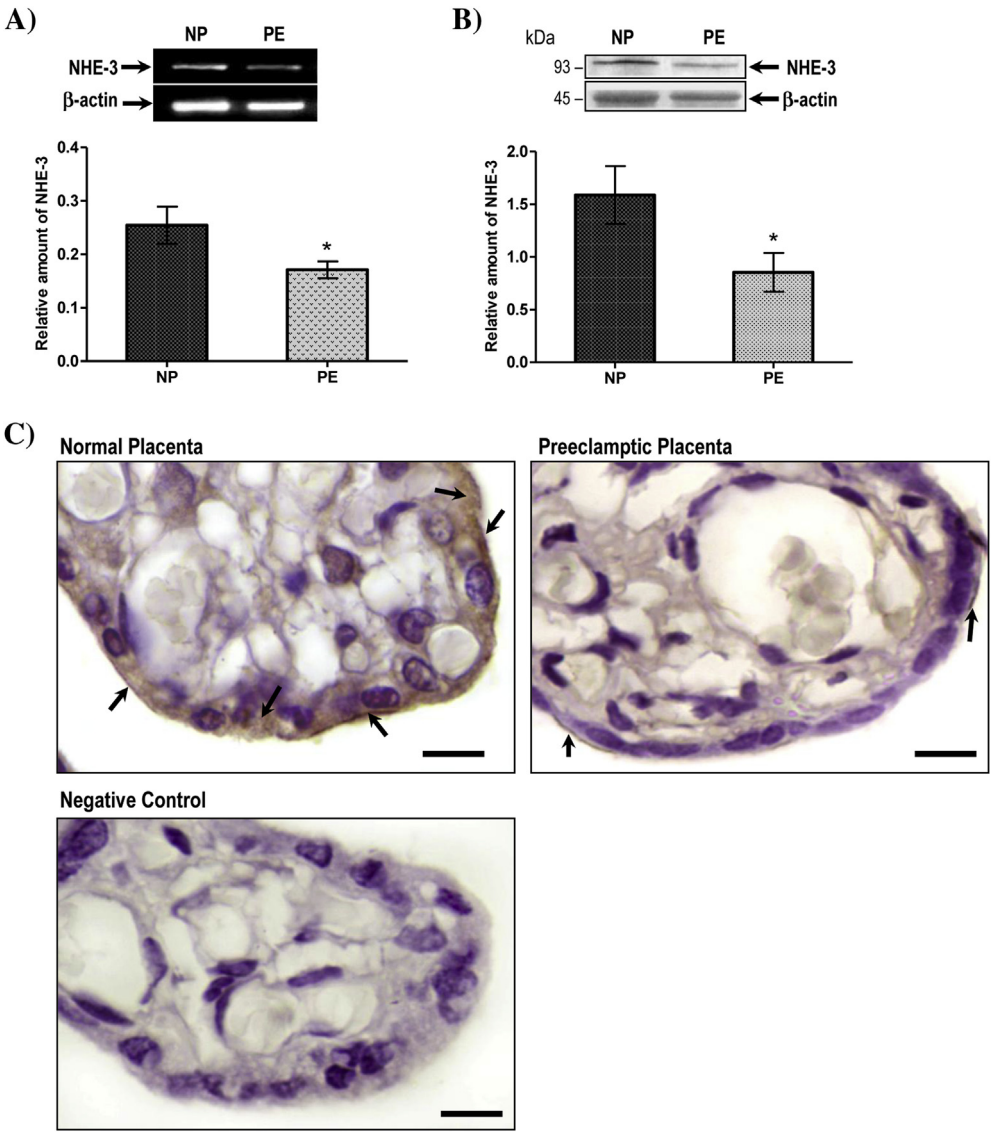
Here, we have examined the mRNA levels, protein expression and localization of the NHE-3 in preeclamptic placentas.

2. Methods

This study was approved by the local ethics committee of the Hospital Nacional Dr. Prof. Alejandro Posadas, Buenos Aires, Argentina, and written consent was obtained from the patients before the collection of samples. Full-term normal ($n = 10$) and preeclamptic ($n = 10$) placental tissues were obtained after cesarean section. Clinical data are shown in Table 1. All women were white hispanic.

Total mRNA was isolated using an SV Total RNA isolation system (Promega Co.) and reverse transcription was performed as previously described [1]. Semi-quantitative RT-PCR was carried out using 5 μM of a specific oligonucleotide primer for human NHE-3 (sense 5′-CTGGACATGCAGTCTCTGGA-3′ and antisense 5′-AGCTTGGTCGACTTGAAGGA-3′). β-actin primers were used as an internal standard. Densitometry of the bands was performed by the ImageJ 1.44 software package.

NHE-3 protein was assessed by Western blot and immunohistochemistry. 75 μg of membrane protein were used for immunoblot studies. After blocking, membranes



were incubated overnight with the primary antibody anti-NHE-3 (Alpha Diagnostic International Inc., 1:500) and then with a goat anti-rabbit immunoglobulin G ([IgG] Jackson ImmunoResearch Laboratories, Inc.; 1:10,000) conjugated to peroxidase. To confirm equal loading, each membrane was also analyzed for β-actin protein expression, showing that the band intensities did not present significant changes between samples. Densitometry was performed and, after normalization with β-actin, the values were plotted as NHE-3/β-actin relative ratio. For localization studies (see Ref. [1] for details) samples were incubated overnight with the primary antibody (1:100). Later, the samples were placed in prediluted link antibody, and incubated in a solution of streptavidin conjugated horse-radish peroxidase. Staining was conducted with Vectastain kit (Vector Laboratories), the labeling was visualized by reaction with DAB (diaminobenzidine tetrahydrochloride), and counterstained with hematoxylin. Control samples were performed by omitting the primary antibody.

3. Results and conclusion

In the present study, we demonstrated that NHE-3 expression was significantly lower in preeclamptic placentas than in normal ones, in both mRNA and protein levels (Fig. 1A & B). Regarding its localization, in normal placentas we observed that the label was located into the apical membrane and abundantly in the cytoplasm of syncytiotrophobast cells. However, in preeclamptic placentas, NHE-3 labeling was weak in the apical membrane and almost undetectable in the cytosol (Fig. 1C).

The inductor mechanisms that cause the decrease NHE-3 expression in preeclamptic placentas are not clear yet.

Most of NHE-3 regulation described in the literature is focused on the acute regulation which is rapid and reversible and often involves changes in phosphorylation, trafficking, and dynamic interaction with regulatory proteins such as NHERFs family [16,17].

In transporting epithelia, such as kidney and intestine, cortisol [18] and aldosterone [19], regulate transcellular sodium transport by modulating not only the activity but also the expression of sodium transport proteins, including NHE-3. In this regard, in human placenta it was found that these steroid hormones may stimulate NHE activity [20], playing a role in the regulation of fetal fluid balance.

On the other hand, chronic regulation of NHE-3 involves transcriptional and translational modifications of NHE-3. In other tissues, it was reported that TNF-α downregulates NHE-3 expression at a transcriptional level [21]. Moreover, it is well known that normal pregnancy stimulates a systemic inflammatory response which is exacerbated in preeclampsia [22]. As it is shown in Table 1, in all the preeclamptic pregnant women whose placentas were used in this work, TNF-α serum levels were higher than in normal ones.

Therefore, the increase in TNF-α together with the decrease in aldosterone [23] might be contributing to the reduce expression of NHE-3 in preeclamptic placentas.

At present, little is known about the function of NHE-3 in human placenta. Our results let us suppose that the decrease in NHE-3 expression might be associated with a reduced NHE-3 activity leading to lower intracellular pH, which may impair nutrient transport and other placental functions. Much further studies are needed to define whether these alterations play a direct role either in the pathogenesis or in the adaptative response of preeclampsia.

Declaration of interest

The author declares that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Fig. 1. A) Semiquantitative RT-PCR and B) semiquantitative western blot analysis of NHE-3 abundance in normal placentas (NP) and preeclamptic placentas (PE). In both experiments we observed that the expression of NHE-3 decreased in PE as compared to normal ones. β-Actin expression was determined to control for unequal loading. Densitometry was performed and after normalization for β-actin, the values of mRNA and protein level expression were plotted as NHE-3/β-actin relative ratio. Each plotted value corresponds to the mean ± SEM ($n = 10$, $^*p < 0.05$). C) Localization of NHE-3 in normal and preeclamptic placentas. Immunostaining with an anti-NHE-3 antibody revealed specific labeling in the apical membrane and abundantly in the cytosol of syncytiotrophoblast cells from normal placentas. In preeclamptic placentas, NHE-3 labeling was weak in the apical membrane and almost undetectable in the cytosol. Negative controls were performed by omitting the primary antibody and replaced by a non-immune rabbit serum. Bar = 10 μm.