



## Short communication

## Expression of the epithelial sodium channel sensitive to amiloride (ENaC) in normal and preeclamptic human placenta

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## ABSTRACT

Aldosterone modulates the activity of the epithelial sodium channel (ENaC) through changes in its trafficking, membrane expression and open probability. Plasma levels of aldosterone are decreased in preeclampsia. Herein we postulated that if aldosterone regulates ENaC expression then its expression should be decreased in preeclampsia. We found a diminished expression of the three subunits of the ENaC in the membranes of preeclamptic placentas in comparison with the normal ones. Although the role of ENaC in placental tissues is poorly understood, these differences may have consequences for the ion transport involved in the pathophysiology of preeclampsia.

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## 1. Introduction

The epithelial sodium channel (ENaC) is highly regulated by aldosterone which is controlled by a negative-feedback by the renin–angiotensin–aldosterone system with aldosterone as the final hormone, essential for  $\text{Na}^+$  balance. Aldosterone modulates ENaC through changes in its three subunits trafficking, membrane expression and open probability ([1,2] for references). Other hormones and paracrine factors such as vasopressin and angiotensin II modulate in parallel the activity of ENaC [3,4].

$\text{Na}^+$  currents are involved in cell migration, documented in tumor cell proliferation [5], epithelial and vascular cells [6,7] and BeWo trophoblastic cells [8]. One of the major extra-renal renin–angiotensin systems during pregnancy is in the placenta, which is altered in preeclampsia [9]. The interaction of genetic, immunological and endocrine factors is involved in the pathophysiology of preeclampsia with inadequate trophoblastic invasion and maternal endothelial dysfunction (for references [10–13]). In preeclampsia there is a lower plasma volume expansion, lower cardiac output and an increase in total peripheral vascular resistance. Plasma levels of renin, angiotensin II and aldosterone increase during normal pregnancy although in preeclampsia for reasons that are unclear, renin activity, angiotensin II, and aldosterone decrease and the ratio aldosterone/renin is increased [10]. We tested the hypothesis that if aldosterone regulates ENaC expression, the amount of ENaC should be decreased in preeclamptic placentas.

## 2. Methods

Membrane vesicles and fixed explants were obtained from normal and preeclamptic placentas at full term, stored at the Cátedra de Biología Celular, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires (see [14] for a detailed description of the cases). ENaC protein was assessed by Western blot or immunohistochemistry [8]. 75  $\mu\text{g}$  of membrane protein from placentas and 100  $\mu\text{g}$  of total proteins from BeWo and T84 cells (positive control) (ATCC, USA) were used for immunoblot studies. We employed rabbit polyclonal antibodies of human  $\alpha\text{ENaC}$  and  $\gamma\text{ENaC}$  (dilution 1:1000 overnight), a mouse monoclonal antibody of human  $\beta\text{ENaC}$  (dilution 1:500 overnight) and a normal rabbit IgG (Santa Cruz Biotechnology Inc, CA, USA) to detect unspecific bands in ENaC blotting from normal placentas. To confirm equal loading, each membrane was stripped and analyzed for actin protein expression, demonstrating that the band intensities did not present significant changes between the samples studied. Densitometry was performed and after normalization with actin, the values were plotted as ENaC/actin relative ratio. For localization studies (see Ref. [8] for details) samples were incubated overnight with the primary antibodies (1:100). Immunofluorescence was performed using anti-rabbit-FITC for detection of  $\alpha$  and  $\gamma\text{ENaC}$  subunits and anti-mouse-FITC for  $\beta\text{ENaC}$  (Jackson Immunoresearch Lab, West Grove, PA, USA) (1:2500, 1 h). Mounted slices were analyzed on a fluorescence microscope (Axiostar HAL 100; Carl Zeiss, Oberkochen, Germany). Control samples were performed by omitting the primary antibody and an additional control for  $\alpha$  and  $\gamma\text{ENaC}$  was carried out with normal rabbit IgG (data not shown).

## 3. Results and discussion

Protein fractions of apical membrane of normal and preeclamptic syncytiotrophoblast were probed with anti- $\alpha$ ,  $\beta$  and  $\gamma\text{ENaC}$  antibodies. The abundance of the subunits of ENaC protein (ENaC/actin relative ratio) from 6 to 8 samples was decreased in preeclamptic placental explants when compared with the normal ones. Similar results were obtained in 6–12 placentas using staining with Ponceau Red to estimate the relative amounts of total protein (data not

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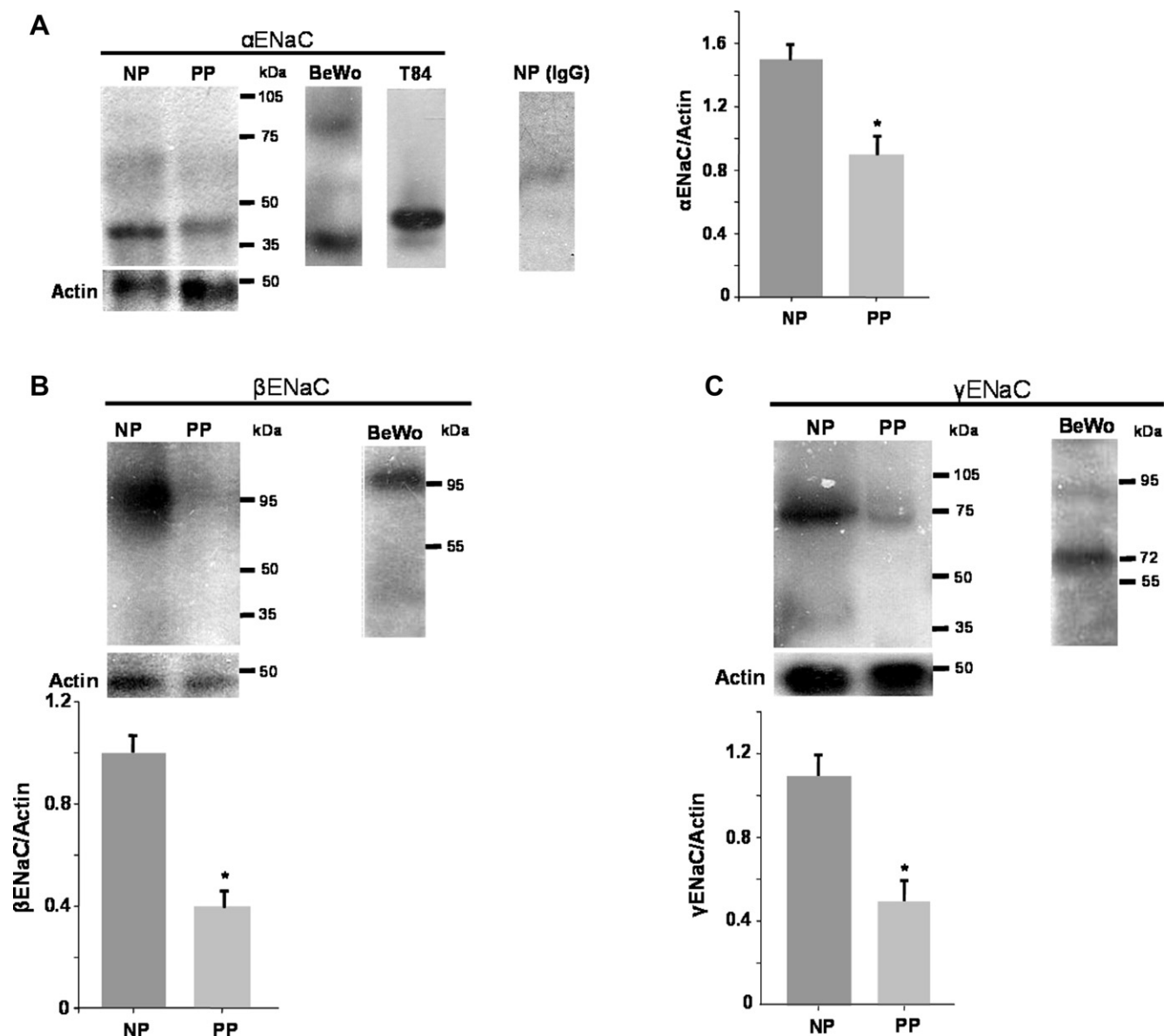
E-mail addresses: [kotsias@retina.ar](mailto:kotsias@retina.ar), [kotsias@yahoo.com](mailto:kotsias@yahoo.com) (B.A. Kotsias).

shown). Fig. 1 shows the proteolytic processed bands of the expected size of ENaC subunits in the cell membrane [8,15]: ~40 kDa for  $\alpha$ ENaC, and ~75 kDa for  $\gamma$ ENaC, and ~100 kDa for  $\beta$ ENaC. A normal rabbit IgG in normal placentas recognized a ~55–60 kDa polypeptide corresponding to a non-specific band. T84 and trophoblastic BeWo cell lines protein extracts were analyzed as positive controls. In addition, we found that the three ENaC subunits are present in the polarized syncytium (Fig. 2A, D, G) but the intensity of the channel protein staining in preeclampsia is lower compared with that in normal samples (B, E, H).

There are reports on the alteration in the expression and properties of ion channels in animal models and human preeclampsia: ENaC [16], potassium and voltage-gated sodium channel [17–19], CFTR [20] and a genetic variant of the  $\beta$ ENaC subunit [21]. ENaCs are activated in a complex manner in response to factors from the external environment, among them a proteolytic processing. Plasmin-dependent cleavage of the  $\gamma$ ENaC subunit [22] may be

particularly relevant in disorders associated with proteinuria and extracellular volume expansion because several years ago Chen et al. [23] found that urine from women with preeclampsia contains an altered plasmin activity depending on the severity of the disease. We detected only a band of 40 kDa of the  $\alpha$ ENaC subunit and a 75 kDa in the  $\gamma$  subunit but not the full-length polypeptides probably because we used only membrane proteins where the proteolytic processed forms of the proteins are necessary for full channel activity [8,15]. We do not have a clear explanation for the difference between the molecular weights of the  $\alpha$ ENaC subunits from samples from membranes of placental tissue (40 kDa) and BeWo cells maintained in culture medium (35 kDa).

The causes of the reduced aldosterone concentration in preeclampsia are not clear. Escher et al. [24] found in preeclamptic women a reduced activity of the synthase CYP11B2 which catalyzes aldosterone although the studies on the association of this gene polymorphism with gestational hypertension or preeclampsia



**Fig. 1.** Semi quantitative immunoblotting analysis of  $\alpha$  (A),  $\beta$  (B) and  $\gamma$  (C) ENaC membrane abundance in normal and preeclamptic placental explants. Representative immunoblots show that ENaC protein level expression from the three subunits was lower in preeclamptic placental explants (PP) than in normal placental explants (NP). Densitometry of immunoblots containing ENaC protein level expression was performed, and after normalization for actin in each lane, the values were plotted as ENaC/actin relative ratio, for all subunits. Each bar corresponds to the mean  $\pm$  SEM obtained from six to eight normal and preeclamptic placental explants ( $p < 0.05$ ). SEM = standard error of mean.

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