Placenta 32 (2011) 1050–1056

Contents lists available at SciVerse ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Evidence for insulin-mediated control of AQP9 expression in human placenta

M. Castro Parodi [a], M. Farina [b], V. Dietrich [a], C. Abán [b], N. Szpilbarg [a], E. Zotta [c], A.E. Damiano [a,*]

[a] Laboratorio de Biología de la Reproducción, Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina
[b] Laboratorio de Fisiopatología Placentaria, CEFyBO-CONICET, Facultad de Medicina, Unversidad de Buenos Aires, Argentina
[c] Laboratorio de Fisiopatogenia, Departamento de Fisiología, Facultad de Medicina, Universidad de Buenos Aires, Argentina

ARTICLE INFO

Article history:
Accepted 30 September 2011

Keywords:
AQP9
Syncytiotrophoblast
Placenta
Insulin
Preeclampsia

ABSTRACT

The AQP9 gene contains a negative insulin response element, suggesting that it may be modulated by insulin. Previously, we reported AQP9 overexpression in preeclamptic placentas but a lack of functionality of AQP9 in water and mannitol transport. We also observed high serum levels of insulin and TNF-α in preeclamptic women.

Objective: To evaluate whether AQP9 expression is regulated by insulin in the human placenta, and whether the dysregulation of AQP9 observed in preeclamptic placentas may be related to the inability to respond to insulin stimuli.

Methods: Explants from normal and preeclamptic placentas were cultured at different concentrations of insulin. Treatment with TNF-α was used to induce phosphorylation of insulin receptor substrate (IRS), which may desensitize insulin action. AQP9 molecular expression and water uptake was determined.

Results: Insulin decreased the molecular expression of AQP9 exclusively in explants from normal placentas in a concentration-dependent manner. Treatment with TNF-α previous to insulin addition prevented these changes. Moreover, insulin treatment did not modify water uptake neither its sensitivity to $HgCl_2$.

Conclusion: AQP9 water permeability seems to be independent of its molecular expression, strongly suggesting that AQP9 might not have a key role in water transport in human placenta. We also propose another mechanism of down-regulation of AQP9 molecular expression mediated by insulin in a concentration-dependent manner in human placenta and provide new evidence that in preeclamptic placentas the mechanisms of insulin signaling may be altered, producing an overexpression of AQP9 that does not correlate with an increase in its functionality.

1. Introduction

During pregnancy, fetal water requirements increase markedly due to an exponential growth in fetal weight. Physiological data indicate that both a transcellular and a paracellular pathway are available for water transfer across the human placenta, but the morphological correlation of the latter is still uncertain [1].

Transcellular water flux may be facilitated by aquaporins (AQPs), a family of small integral membrane proteins (30-kDa monomers) that transport either water alone or water and small solute(s) such as glycerol [2,3].

In mammals, there are at least 13 AQPs, which show a wide range of distribution in organs that are actively involved in water movement.

Aquaporin 9 (AQP9) is a member of the aquaglyceroporin subfamily of AQPs and shares the highest amino acid sequence homology with AQP3, AQP7, and AQP10 [4,5]. In addition to water, AQP9 transports small uncharged molecules like glycerol, urea, purines, and pyrimidines but its physiological function(s) remains unknown.

In human placenta, we localized AQP9 in the apical membrane of the syncytiotrophoblast (hST) and hypothesized that AQP9 may participate in the water transport between the mother and the fetus [6]. In subsequent experiments in preeclamptic placentas, we observed an increase in AQP9 expression and a different cellular distribution of the protein [7]. We expected that the increase in AQP9 could be correlated with an increase in water flux. In contrast, we found that the uptake of water and mannitol in preeclamptic

* Corresponding author. Cátedra de Biología Celular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956 1er piso, CP 1113, Buenos Aires, Argentina.
E-mail address: adamiano@ffyb.uba.ar (A.E. Damiano).

0143-4004/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2011.09.022

placental explants decreased when compared to those observed in normal ones. We also found that the uptake of water was not sensitive to $HgCl_2$, a general inhibitor of AQPs, suggesting a lack of functionality of this protein for water transport. However, the possible roles of AQP9 in the human placenta are still speculative.

Little is known about AQP9 regulation. Recently, we have reported that hormones such as human chorionic gonadotropin (hCG) secreted by villous trophoblasts may induce the expression of AQP9 [8]. We demonstrated that increased serum levels of hCG may be involved in the increased AQP9 protein expression in preeclamptic placentas via adenosine 3(‘),5(‘)-cyclic phosphate pathways. However, the presence of numerous sites of regulation in the gene and on the protein suggests that other mechanisms may be involved in the regulation of AQP9 expression.

It is generally accepted that in addition to stimulating glucose transport [9,10], the binding of insulin to cell surface receptors alters the expression of numerous genes in a variety of tissues [11]. In placental and trophoblastic cells, insulin has no stimulatory effect on glucose uptake or glycogen synthesis [12,13], but is involved in the enhancement of the synthesis of human placental lactogen (hPL) [14] and hCG [15] and in the regulation of 3β-hydroxysteroid dehydrogenase [16]. Although insulin primarily regulates gene expression at the transcriptional level, the detailed molecular mechanism through which it mediates these effects in the placenta remains unknown. It has been recently reported in mice that insulin represses the expression of adipose AQP7 and hepatic AQP9 through negative insulin response elements (IRE) in their promoter genes [17,18]. Non-rodent species, such as pig [19], also show IRE in the AQP9 gene promoter, whereas potential IRE can also be found in the human AQP9 gene [5]. Thus, alterations in insulin signaling are intimately related to AQPs expression at a molecular level.

Although insulin resistance and the resultant hyperinsulinemia are features of normal pregnancy and are maximal in the third trimester, pregnant women complicated by preeclampsia have exaggerated insulin resistance associated with other metabolic alterations [20–23].

Furthermore, it is well known that normal pregnancy stimulates a systemic inflammatory response which is exacerbated in preeclampsia [24]. Proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), are highly expressed in preeclampsia and may also be involved in the insulin resistance associated with this syndrome [25,26].

Recently, Scioscia and co-workers have reported placental-specific down-regulation of insulin signaling in preeclampsia, associated with the inhibitory effect of insulin receptor substrate (IRS) −1 and −2 by means of serine phosphorylation [27]. In this regard, they proposed that the inactivation of IRS in placental tissues of preeclamptic women can occur as a consequence of the systemic inflammation via TNF-α. However, it is still not clear whether this abnormal finding is due to immunological dysfunction or chronic hyperinsulinemia.

Since the relationship between AQP9 and insulin in human placenta has not been explored, here we postulate that insulin might be involved in the down-regulation of AQP9 in the human placenta, and that the dysregulation of AQP9 observed in preeclamptic placentas may be related to the inability to respond to insulin stimuli.

2. Materials and methods

2.1. Tissue collection

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 ($n = 12$) and preeclamptic ($n = 12$) placental tissues were obtained after cesarean section and blood samples were obtained at admission by venipuncture. Clinical data are shown in Table 1. All women were white hispanic.

Insulin, β-hCG, IGF-1 and TNF-α were measured in the serum.

2.2. Tissue culture

Placental tissue was gently separated by sterile dissection from different cotyledons, excluding chorionic and basal plates, minced with scalpel blades, and washed repeatedly with 0.9% sodium chloride to remove blood from the intervillous space. Whole villous tissue (~50 mg/well) was incubated in 24-well polystyrene tissue culture dishes in 2 mL of serum-free Dulbecco modified Eagle medium (DMEM; Life Technologies, Inc.) containing 100 IU/mL penicillin, 100 mg/mL streptomycin, 32 mg/mL gentamicin at 37 °C for 24 h in a humidified gas mixture of 5% CO_2 and 95% air [7,28].

The concentrations of human recombinant insulin (Humulin R, Lilly Co) added were 1, 10, and 100 μIU/mL. Some explants were incubated with 5 pg/mL TNF-α for 2 h previous to the insulin treatment. To block the effect of TNF-α was used 1 μg/mL etarnercept (Enbrel®, Pfizer Inc, USA) a dimeric fusion protein which mimics the inhibitory effects of soluble TNF receptors.

2.3. Explant viability

Explant viability was verified by the release of the intracellular enzyme lactate dehydrogenase (LDH) into the incubation medium after 24 h of incubation. LDH concentrations were determined with a Lactate Dehydrogenase Assay kit (Sigma–Aldrich Corp), according to the manufacturer's protocol.

2.4. Semiquantitative RT-PCR

Total RNA was isolated using an SV Total RNA isolation system (Promega Co) and reverse transcription was performed as previously described [6]. PCR was carried out using 5 μM of a specific oligonucleotide primer designed on the basis of a highly conserved region flanked by Asn-Pro-Ala (NPA) in the aquaporin family (sense 5′-CATCAACCCAGCTGTGTCT-3′, antisense 5′-CAGCCACTGTTCAGTCCCA-3′), amplifying a 393-bp fragment of human AQP9 [6]. β-actin primers were used as internal standard. Densitometry of the bands was performed by the ImageJ 1.44 software package.

2.5. Immunoblotting

Treated and untreated explants from normal term placentas were processed according to the method previously described [6]. For immunoblotting studies, 100 μg of membrane fraction proteins was dissolved in loading buffer (4% sodium dodecyl sulfate, 0.125 mol/L Tris-HCl pH 6.8, 0.2 mol/L dithiothreitol, 0.02% bromophenol blue, 20% glycerol), heated to 90 °C for 2 min, resolved on 15% polyacrylamide gel, and electrotransferred onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech Ltd). After blocking, membranes were incubated overnight with the primary antibody anti-AQP9 (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.

Table 1
Clinical characteristics of preeclamptic and normotensive women. Values are mean ± SD. Preeclampsia was defined as systolic blood pressure ≥170 mm Hg and/or diastolic pressure ≥110 mm Hg, with proteinuria ≥0.3 g/day or 2 pluses on urine dipstick after the 20th week of gestation in a previously normotensive patient.

	Normotensive women	Preeclamptic women
Number of pregnant women	12	12
Parity		
Primiparous	5	6
Multiparous	7	6
Maternal age, yr	21.7 ± 1.4	25.4 ± 2.5
Gestational age, wk	38.9 ± 1.1	36.7 ± 0.7
Mean blood pressure, mm Hg		
Systolic	110 ± 4.0*	160.0 ± 5.0*
Diastolic	63 ± 2.5**	112.0 ± 1.8**
Proteinuria	negative	+++
Body Mass index (BM), kg/m^2	25 ± 4	24 ± 3
Birth weight, g	3120 ± 230	2780 ± 270
Fetal sex		
Male	6	7
Female	6	5

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

Immunoreactivity was detected using the Enhanced Chemiluminescence (ECL) Western Blotting Analysis System (ECL plus, Amersham Pharmacia Biotech Ltd) according to the manufacturer's instructions.

Photographs of the membranes were taken using a digital camera and analyzed using the ImageJ 1.44 software package.

To confirm equal loading, each membrane was stripped and analyzed for β-actin protein expression, demonstrating that the band intensities did not present significant changes between the samples studied. Briefly, membranes were incubated overnight with monoclonal anti-β-actin (Alpha Diagnostic International Inc) diluted 1:2000, and then revealed as described above.

2.6. Immunoperoxidase and immunoflurescence

Treated and untreated explants were fixed overnight in 10% formaldehyde-0.1 mol/L sodium phosphate buffer (PBS), pH 7.4, dehydrated, and embedded in paraffin as we have previously described [6]. Then, thin sections (4–5 mm) were cut, dewaxed, rehydrated and incubated in 3% hydrogen peroxide (H_2O_2)/methanol for 5 min to block endogenous peroxidase. After blocking nonspecific binding sites with DAKO reagent (DAKO LSAB kit, Dako Corp), tissues slices were incubated overnight (4 °C) with anti-AQP9 (1:50). 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) and labeling was visualized by reaction with DAB (diaminobenzidine tetrahydrochloride) and counterstained with hematoxylin.

For immunofluorescence experiments [28], after incubation with the primary antibody, tissue slices were treated with anti-rabbit IgG antibody conjugated with fluorescein (dilution 1:400) (Santa Cruz Biotechnology) and the labeling was observed in an epifluorescent microscope (Nikon, Eclipse E200) provided with green filter.

Negative controls were performed by omitting the primary antibody.

2.7. Water uptake

Placental explants were incubated at room temperature, in 0.5 mL Ringer's solution containing ^{3}HOH [New England Nuclear Corp]. The uptake was stopped after different times, by adding ice-cold Ringer solution. At the end of the incubations, explants were quickly washed in cold Ringer's solution and solubilized with 1.0 ml of 1 M sodium hydroxide overnight at 37 °C. Aliquots of the solubilized explants were vortex-mixed with 2.0 ml of scintillant (Optiphase "HiSafe", Wallc Oy) and counted on a scintillation counter [7,28]. Other aliquots were kept for determination of protein concentration by the BCA assay (Pierce), according to the manufacturer's protocol.

Inhibition studies with a mercurial compound were carried out in placental explants previously incubated for 10 min in Ringer solution containing 0.3 mM $HgCl_2$. Solute uptake experiments were then performed as described above.

The uptake data ($pmol \cdot mg^{-1} \cdot min^{-1}$) obtained from each group were compared by one-way analysis of variance (ANOVA test) followed by Fisher LSD test.

2.8. Statistical analysis

All statistical analysis was carried out with *Statistica software version 6.0* (Statistica, StatSoft, Inc Tulsa). The criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Increased insulin concentrations in preeclamptic sera

The demographic and clinical characteristics of the subjects studied are presented in Table 1. All women belonged to the white Hispanic ethnic group. We observed an increase in the concentrations of insulin and TNF-α, with no changes in IGF-1, as assessed in the sera from both groups of patients (Table 2).

Table 2
β-hCG, serum insulin, IGF-1 and TNF-α levels in preeclamptic and normotensive women.

	Normotensive women	Preeclamptic women
β-hCG (IU/mL)	11.93 ± 2.33	31.68 ± 7.42*
Insulinemia (μIU/mL)	8.1 ± 1.0	18.1 ± 6.4*
TNF-α (pg/mL)	9.6 ± 1.3	15.3 ± 0.4*
IGF-1 (mg/dL)	200.9 ± 30.7	254.5 ± 28.7

*$P < 0.01$.

3.2. Explant viability

Insulin treatment of placental villous explants from normal and preeclamptic placentas did not decrease villous explant viability, as measured by the release of LDH ($p > 0.05$ compared to untreated controls).

3.3. Insulin effect on AQP9 expression in normal and preeclamptic placental explants

Next, we evaluated the effect of insulin on AQP9 protein expression in explants from normal and preeclamptic placentas.

In normal placental explants not treated with insulin, a 37-kDa band corresponding to the glycosylated form of AQP9 was observed. However, this band was weakly detected when the explants were treated with 100 μUI/mL insulin.

In preeclamptic placentas, to determine if the lack of response to insulin repression was due to defects in the hormone or changes in the receptor, we incubated preeclamptic explants with 100 μUI/mL insulin. In these explants, insulin treatment did not modify AQP9 expression and a smaller band of 28-kDa, corresponding to the unglycosylated form of the protein, was also detected (Fig. 1).

3.4. Concentration-dependent effect of insulin on AQP9 expression

To determine whether the effect of insulin on AQP9 expression depends on insulin concentration, we cultured normal placental explants with different concentrations of insulin.

Insulin decreased the expression of AQP9 mRNA in explants from normal placentas in a concentration-dependent manner (Fig. 2A).

The results of immunoblotting are shown in Fig. 2B, where a concentration-dependent decrease in AQP9 protein is clear in the presence of different concentrations of insulin.

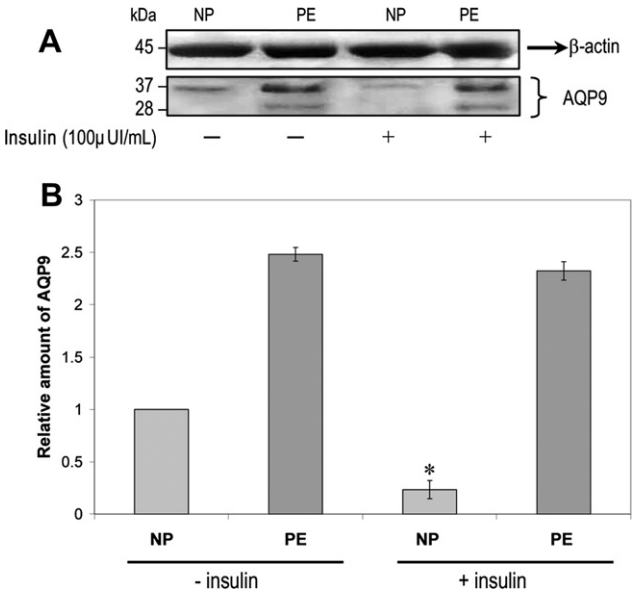

Fig. 1. Insulin effect on AQP9 expression in normal (NP) and preeclamptic placental (PE) explants. A) A representative immunoblot shows a 37-kDa band corresponding to the glycosylated form of AQP9 in normal placental explants not treated with insulin. After insulin treatment, AQP9 protein expression decreased 3.2-fold. However, in preeclamptic explants, insulin treatment had no effect on AQP9 expression. B) Densitometry of immunoblots containing AQP9 protein level expression was performed, and after normalization for β-actin, the values were plotted as AQP9/β-actin relative ratio. Each plotted value corresponds to the mean ± SEM obtained from 12 normal and 12 preeclamptic placental explants ($p < 0.05$).

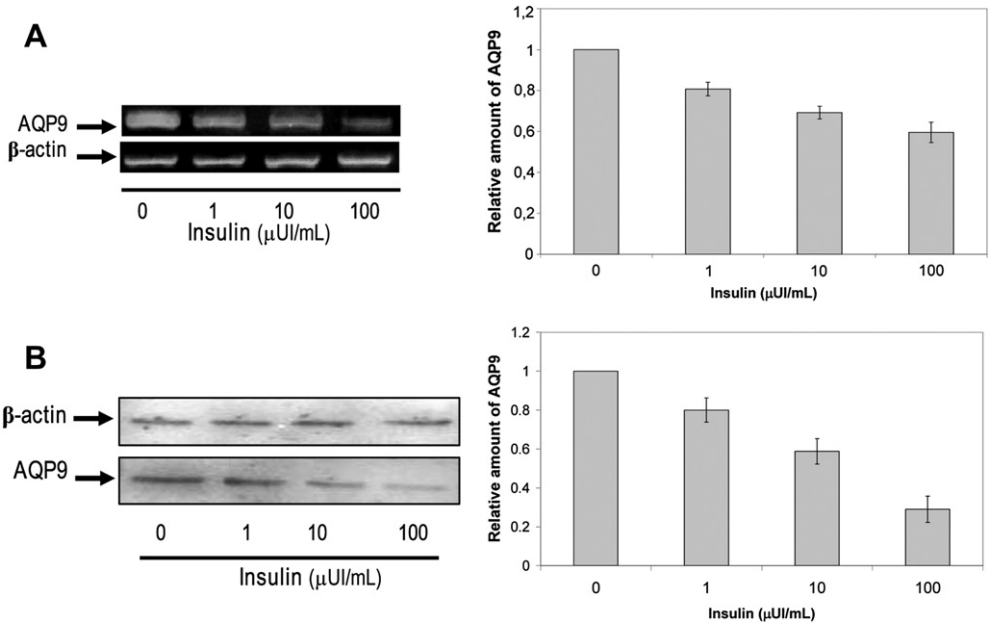

Fig. 2. Concentration-dependent effect of insulin on AQP9 expression. Normal placental explants were treated with 1, 10 and 100 μUI/mL insulin. Semiquantitative RT-PCR and Western blot were performed. Densitometry was performed and after normalization for β-actin, the values were plotted as AQP9/β-actin relative ratio. A) Semiquantitative RT-PCR and B) semiquantitative Western blot showed that insulin treatment down-regulated the expression of AQP9 in explants from normal placentas in a concentration-dependent manner ($p < 0.05$, $n = 12$).

In agreement with the immunoblotting assay, immunofluorescence studies showed that AQP9 signal was almost undetectable after 100 μUI/mL insulin treatment. Moreover, immunohistochemistry studies revealed a faint AQP9 labeling in the apical membrane of hST after the addition of insulin (Fig. 3B).

3.5. TNF-α effect on AQP9 expression in normal placental explants

To evaluate whether the inability of AQP9 to respond to insulin stimuli in preeclamptic placentas is related to the increase in TNF-α, normal placental explants were cultured with 5 pg/mL TNF-α previous to 100 μUI/mL insulin treatment. We observed that TNF-α alone did not modify AQP9 expression and the subsequent addition of insulin did not reduce AQP9 expression and showed the same expression pattern as controls (Fig. 4A). Furthermore, we also treated explants with 5 pg/mL TNF-α and 1 μg/mL etanercept, a fusion protein which mimics the effect of soluble TNF-α receptors blocking TNF-α action. In this case, we observed that AQP9 expression was reduced significantly after the adding of insulin, confirming a possible role of this cytokine in impairing insulin signaling.

3.6. AQP9 functionality

After having established the cellular viability of the placenta explants, we determined water uptake. Interestingly, we observed that although the expression of AQP9 decreased dramatically with the increase in insulin concentration, insulin treatment did not modify water uptake or its sensitivity to $HgCl_2$. In addition, TNF-α treatments showed the same results (Fig. 5).

4. Discussion

It is well known that normal pregnancy is characterized by insulin resistance [29]. However, the molecular mechanisms involved in this insulin resistance state are still incompletely characterized. Besides, preeclampsia has been reported to be associated with marked hyperinsulinemia [30,31] and several observations have suggested the possibility that insulin resistance may be involved in its pathogenesis [23].

Emerging evidence shows that, under physiological conditions, insulin regulates the expression of several genes, including the AQP9 gene in the liver and brain [17,32,33]. However, the relationship between insulin and placental AQP9 has not yet been examined. AQP9 is of special interest because, in addition to being permeable to water, it is permeable to neutral solutes. AQP9 is highly expressed in hST from normal placentas and shows an overexpression in preeclamptic placentas, with a lack of functionality for water transport [6,7].

Here, we quantified the insulin serum levels in normotensive and preeclamptic pregnant women and found that insulin levels in preeclamptic women were higher than in normotensive ones. In agreement with our previous report, the basal expression of AQP9 in these preeclamptic placentas was 2.5-fold higher than in normal ones [7]. This result led us to speculate that AQP9 might be regulated by insulin in human placenta.

Thus, we cultured normal placental explants with different concentrations of insulin. We observed that insulin has a concentration-dependent inhibitory effect on AQP9 expression in normal placentas, suggesting that AQP9 expression is either directly or indirectly down-regulated by insulin.

Therefore, we next wondered what may be the situation in preeclamptic placentas.

We performed the same experiment using preeclamptic placental explants and found that insulin treatment did not modify AQP9 expression. Consequently, we speculated that in preeclamptic placentas a disturbed insulin signaling could explain, at least in part, the poor response of AQP9 to the insulin stimuli. Increasing evidence shows that in preeclampsia, the exacerbation of insulin resistance may lead to an impaired insulin signal with low activation of IRS-1 (through multiple tyrosine phosphorylations). Moreover, it was also found that the phosphorylation of serine residues

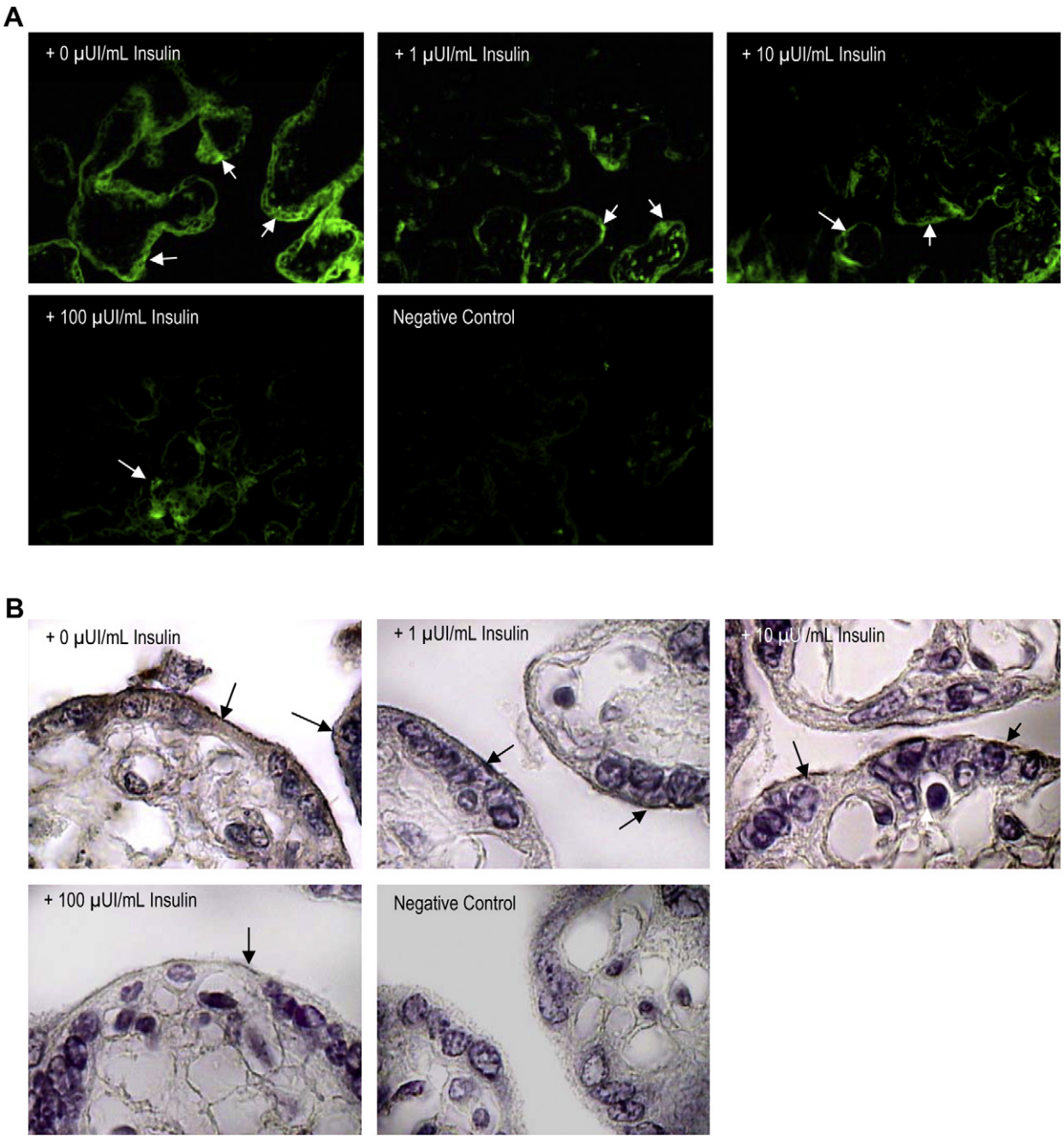

Fig. 3. Localization of AQP9 proteins in hST from normal placental explants treated with different concentrations of insulin. A) Immunofluorescence assay showed that AQP9 signal was almost undetectable after 100 µUI/mL insulin treatment. Magnification: ×400. B) Immunostaining with an anti-AQP9 antibody revealed specific labeling in the apical membrane of hST from normal placentas. This labeling was almost undetectable after the addition of 100 µUI/mL insulin. Negative controls were performed by omitting the primary antibody and replaced by a non-immune rabbit serum. Magnification: ×1000.

in both IRS-1 and IRS-2 results in desensitization of insulin action [34,35]. Although, under physiological conditions insulin can use this negative-feedback mechanism to terminate its own action; it may be activated chronically by the hyperinsulinemia observed in preeclampsia [27].

It is well established that the excessive inflammatory response further exacerbates insulin resistance in several tissues [34–37]. In addition, there is growing evidence that the relationship between inflammation and insulin resistance is not merely correlative, but actually causative [38,39]. It has also been reported that the inactivation of IRS in placental tissues of preeclamptic women can occur as a consequence of the systemic inflammation via TNF-α [27]. TNF-α was proposed to impair insulin signaling at the level of the IRS proteins. In this regard, the Ser(307) residue in IRS-1 has been identified as a site for the inhibitory effects of TNF-α. Kanety and co-workers have demonstrated that TNF-α leads to a reduction in insulin-induced tyrosine phosphorylation of IRS-1 through increased serine phosphorylation of the substrate. This increased serine phosphorylation interferes with insulin-induced tyrosine phosphorylation of IRS-1 and impairs insulin action [40].

In this regard, we observed that in all the preeclamptic women, who have an increase in insulin levels, TNF-α concentrations were

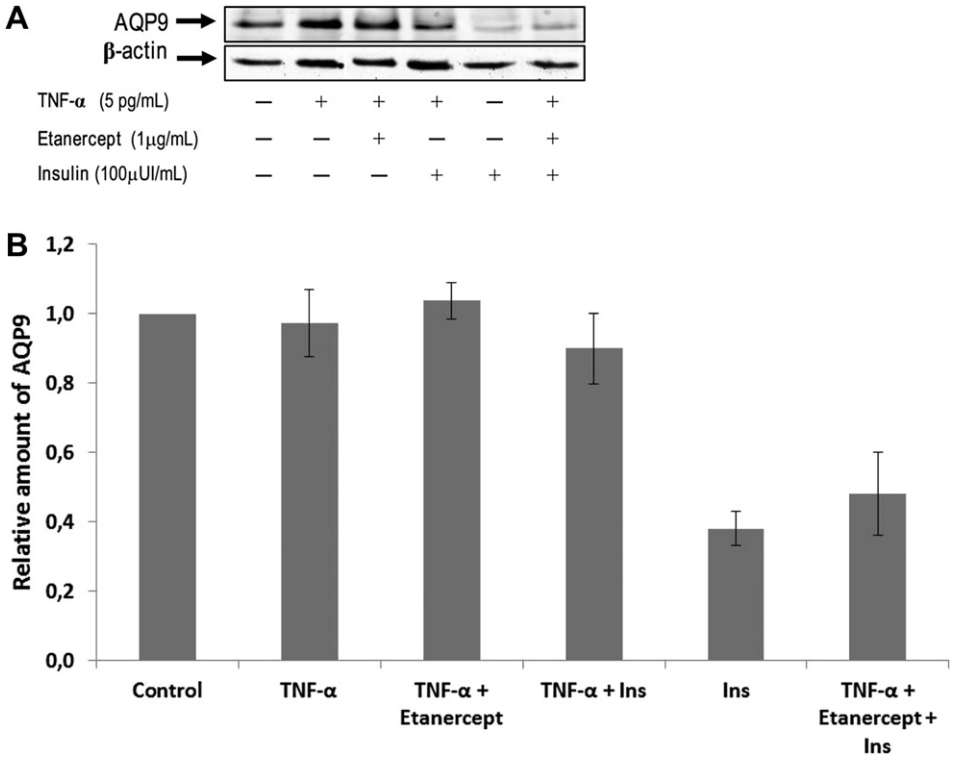

Fig. 4. TNF-α effect on AQP9 expression. A) A representative Western blot analysis revealed TNF-α alone did not modify AQP9 expression and the subsequent addition of 100 μUI/mL insulin did not reduce AQP9 expression and showed the same expression pattern as controls. However, in explants treated with 5 pg/mL TNF-α and 1 μg/mL etanercept, a fusion protein which blocks TNF-α action, AQP9 expression was reduced significantly after the adding of insulin. B) Densitometry of immunoblots containing AQP9 protein level expression was performed, and after normalization for β-actin, the values were plotted as AQP9/β-actin relative ratio ($p < 0.05$).

higher than in normal pregnant women. In order to distinguish the role of TNF-α, we examined the effect of this cytokine on insulin signaling. We found that AQP9 expression was not modified by insulin in explants previously treated with TNF-α. On the other hand, insulin was able to down-regulate AQP9 expression after blocking TNF-α action by etanercept. These data provide new evidence that this pro-inflammatory cytokine is involved in the desensitization of insulin action.

Although, the hyperinsulinemic state during preeclampsia may probably justify the reduced insulin response of AQP9, it cannot explain AQP9 overexpression. Our hypothesis is that the increased expression of AQP9 in preeclamptic placenta is consistent with a synergist effect of the inability of insulin to down-regulate AQP9 and others factors such as hCG which are involve in the upregulation of the protein [8].

As regards the functional experiments, we expected that the decrease in AQP9 expression correlated with a decrease in water flux after the addition of insulin. However, here we found that in normal placental explants treated with insulin, water uptake was similar to that observed in non-treated explants, and that it was also sensitive to $HgCl_2$. Unexpectedly, the decrease in the molecular expression of AQP9 showed no liaison with the functional

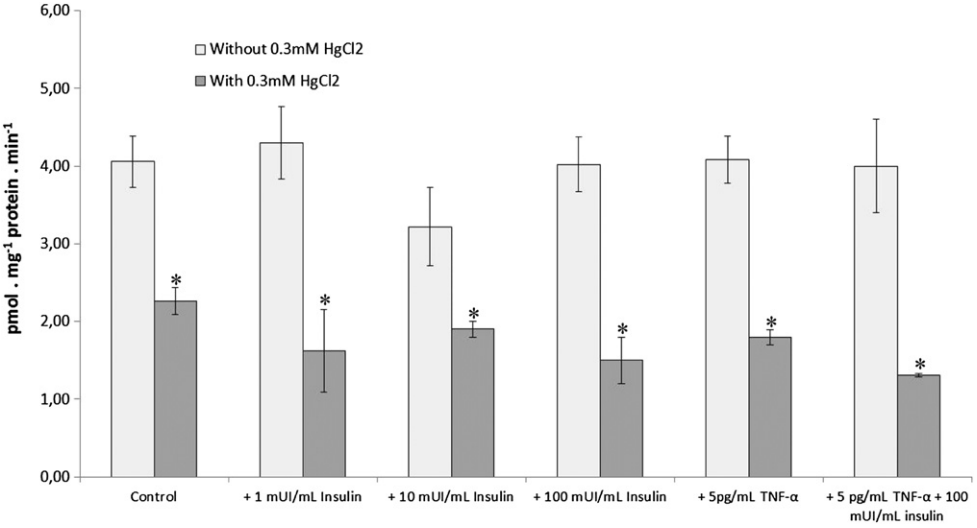

Fig. 5. Water uptake in explants from insulin-treated and non-treated normal term placentas. Explants from normal term placentas ($n = 12$) and were cultured with 1, 10 or 100 μUI/ml of insulin, and with and without TNF-α. In all cases, water uptake was similar to the control and sensitive to $HgCl_2$ ($P < 0.01$).

experiments, suggesting that water is passing through other AQPs, such AQP3 or AQP4 [6,41]. These results are consistent with those observed in preeclamptic placentas where the increased expression of AQP9 was not related to the water transport [7]. Therefore, we speculated that AQP9 was not exclusively involved in water transport between the mother and the fetus [42] and might be involved in the diffusion of metabolites such as glycerol, and therefore have a main role in energy metabolism, analogously to that observed in the liver and brain [17,32,33].

In conclusion, this is the first report in which it is shown that AQP9 water permeability seems to be independent of its molecular expression, strongly suggesting that AQP9 might not have a key role in water transport in human placenta.

Moreover, we propose another mechanism of down-regulation of AQP9 molecular expression mediated by insulin in a concentration-dependent manner in human placenta. Our work provides new evidence that in preeclamptic placentas the mechanisms of insulin signaling may be altered, producing an overexpression of AQP9 that does not correlate with an increase in its functionality.

Finally, although the physiological importance of AQP9 is still under study, the expression of this protein appears to have several points of regulation in the human placenta.

Therefore, we may speculate that AQP9 dysregulation might have a central role in the physiopathogenesis of preeclampsia.

Declaration of interest

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

Acknowledgments

We thank Dr. Bernardo Maskin for his help in obtaining placental tissue. This study was supported by UBACyT B603 and ANCyPT 1014 grants.

References

[1] Edwards DC, Jones J, Sibley CP, Nelson DM. Paracellular permeability pathways in the human placenta: a quantitative and morphological study of maternal-fetal transfer of horseradish peroxidase. Placenta 1993;14(1):63–73.
[2] Agre P, Bonhivers M, Bornia MJ. The aquaporins, blueprints for cellular plumbing systems. J Biol Chem 1998;273(24):14659–62.
[3] Carbrey JM, Agre P. Discovery of the aquaporins and development of the field. Handb Exp Pharmacol 2009;190:3–28.
[4] Ishibashi K, Kuwahara M, Kageyama Y, Tohsaka A, Marumo F, Sasaki S. Cloning and functional expression of a second new aquaporin abundantly expressed in testis. Biochem Biophys Res Commun 1997;237(3):714–8.
[5] Tsukaguchi H, Shayakul C, Berger UV, Mackenzie B, Devidas S, Guggino WB, et al. Molecular characterization of a broad selectivity neutral solute channel. J Biol Chem 1998;273(38):24737–43.
[6] Damiano AE, Zotta E, Goldstein J, Reisin I, Ibarra C. Water channel proteins AQP3 and AQP9 are present in syncytiotrophoblast of human term placenta. Placenta 2001;22(8–9):776–81.
[7] Damiano AE, Zotta E, Ibarra C. Functional and molecular expression of AQP9 channel and UT-A transporter in normal and preeclamptic human placentas. Placenta 2006;27(11–12):1073–81.
[8] Marino GI, Castro-Parodi M, Dietrich V, Damiano AE. High levels of human chorionic gonadotropin (hCG) correlate with increased aquaporin-9 (AQP9) expression in explants from human preeclamptic placenta. Reprod Sci 2010;17(5):444–53.
[9] Suzuki K, Kono T. Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 1980;77(5):2542–5.
[10] Forest CD, O'Brien RM, Lucas PC, Magnuson MA, Granner DK. Regulation of phosphoenolpyruvate carboxykinase gene expression by insulin, use of the stable transfection approach to locate an insulin responsive sequence. Mol Endocrinol 1990;4(9):1302–10.
[11] O'Brien RM, Granner DK. Regulation of gene expression by insulin. Biochem J 1991;278(4):609–19.
[12] Challier JC, Hauguel S, Desmaizieres V. Effect of insulin on glucose uptake and metabolism in the human placenta. J Clin Endocrinol Metabm 1986;62(5):803–7.
[13] Schmon B, Hartmann M, Jones CJ, Desoye G. Insulin and glucose do not affect the glycogen content in isolated and cultured trophoblast cells of human term placenta. J Clin Endocrinol Metab 1991;73(4):888–93.
[14] Hochberg Z, Perlman R, Brandes JM, Benderli A. Insulin regulates placental lactogen and estradiol secretion by cultured human term trophoblast. J Clin Endocrinol Metab 1983;57(6):1311–3.
[15] Ren SG, Braunstein GD. Insulin stimulates synthesis and release of human chorionic gonadotropin by choriocarcinoma cell lines. Endocrinology 1991;128(3):1623–9.
[16] Nestler JE. Insulin and insulin-like growth factor-I stimulate the 3 beta-hydroxysteroid dehydrogenase activity of human placental cytotrophoblasts. Endocrinology 1989;25(4):2127–33.
[17] Kuriyama H, Shimomura I, Kishida K, Kondo H, Furuyama N, Nishizawa H, et al. Coordinated regulation of fat-specific and liver-specific glycerol channels, aquaporin adipose and aquaporin 9. Diabetes 2002;51(10):2915–21.
[18] Kishida K, Shimomura I, Kondo H, Kuriyama H, Makino Y, Nishizawa H, et al. Genomic structure and insulin-mediated repression of the aquaporin adipose (AQPap), adipose-specific glycerol channel. J Biol Chem 2001;276(39):36251–60.
[19] Li X, Lei T, Xia T, Chen X, Feng S, Chen H, et al. Molecular characterization, chromosomal and expression patterns of three aquaglyceroporins (AQP3, 7, 9) from pig. Comp Biochem Physiol B Biochem Mol Biol 2008;149(3):468–76.
[20] Wolf M, Sandler L, Muñoz K, Hsu K, Ecker JL, Thadhani R. First trimester insulin resistance and subsequent preeclampsia: a prospective study. J Clin Endocrinol Metab 2002;87(4):1563–8.
[21] Belo L, Caslake M, Gaffney D, Santos-Silva A, Pereira-Leite L, Quintanilha A, et al. Changes in LDL size and HDL concentration in normal and preeclamptic pregnancies. Atherosclerosis 2002;162(2):425–32.
[22] Ogura K, Miyatake T, Fukui O, Nakamura T, Kameda T, Yoshino G. Low-density lipoprotein particle diameter in normal pregnancy and preeclampsia. J Atheroscler Thromb 2002;9(1):42–7.
[23] Kaaja R, Tikkanen MJ, Viinikka L, Ylikorkala O. Serum lipoproteins, insulin, and urinary prostanoid metabolites in normal and hypertensive pregnant women. Obstet Gynecol 1995;85(3):353–6.
[24] Redman CW, Sargent IL. Preeclampsia and the systemic inflammatory response. Semin Nephrol 2004;24(6):565–7.
[25] Visser W, Beckmann I, Knook MA, Wallenburg HC. Soluble tumor necrosis factor receptor II and soluble cell adhesion molecule 1 as markers of tumor necrosis factor-alpha release in preeclampsia. Acta Obstet Gynecol Scand 2002;81(8):713–9.
[26] Vince GS, Starkey PM, Austgulen R, Kwiatkowski D, Redman CW. Interleukin-6, tumour necrosis factor and soluble tumour necrosis factor receptors in women with pre-eclampsia. Br J Obstet Gynaecol 1995;102(1):20–5.
[27] Scioscia M, Gumaa K, Kunjara S, Paine MA, Selvaggi LE, Rodeck CH, et al. Insulin resistance in human preeclamptic placenta is mediated by serine phosphorylation of insulin receptor substrate-1 and -2. J Clin Endocrinol Metab 2006;91(2):709–17.
[28] Castro-Parodi M, Levi L, Dietrich V, Zotta E, Damiano AE. CFTR may modulate AQP9 functionality in preeclamptic placentas. Placenta 2009;30(7):642–8.
[29] Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. Am J Obstet Gynecol 1991;165(6):1667–72.
[30] Fuh MM, Yin CS, Pei D, Sheu WH, Jeng CY, Chen YI, et al. Resistance to insulin-mediated glucose uptake and hyperinsulinemia in women who had preeclampsia during pregnancy. Am J Hypertens 1995;8(7):768–71.
[31] Martinez AE, Gonzalez OM, Quinones GA, Ferrannini E. Hyperinsulinemia in glucose-tolerant women with preeclampsia. A controlled study. Am J Hypertens 1996;9(6):610–4.
[32] Carbrey JM, Gorelick-Feldman DA, Kozono D, Praetorius J, Nielsen S, Agre P. Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver. Proc Natl Acad Sci USA 2003;100(5):2945–50.
[33] Badaut J, Regli L. Distribution and possible roles of aquaporin 9 in the brain. Neuroscience 2004;129(4):971e81.
[34] Gual P, Marchand-Brustel Y, Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie (Paris) 2005;87:99.
[35] Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, et al. Insulin/IGF-1 and TNF-α stimulate phosphorylation of IRS-1 at inhibitory Ser307 via distinct pathways. J Clin Invest 2001;107:181–9.
[36] White MF. Insulin signaling in health and disease. Science 2003;302:1710–1.
[37] Anim-Nyame N, Sooranna SR, Jones J, Alaghband-Zadeh J, Steer PJ, Johnson MR. Insulin resistance and pre-eclampsia: a role for tumor necrosis factor-alpha? Gynecol Endocrinol 2004;18(3):117–23.
[38] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995;95(5):2409–15.
[39] Lin SY, Wang YY, Sheu WH. Increased serum soluble tumor necrosis factor receptor levels are associated with insulin resistance in liver cirrhosis. Metabolism 2004;53(7):922–6.
[40] Kanety H, Feinstein R, Papai MZ, Hemi R, Karasik M. Tumor necrosis factor α-induced phosphorylation of InsulinReceptor Substrate-1 (IRS-1). J Biol Chem 1995;270(40):23780–4.
[41] De Falco M, Cobellis L, Torella M, Acone G, Varano L, Sellitti A, et al. Down-regulation of aquaporin 4 in human placenta throughout pregnancy. In Vivo 2007;21(5):813–7.
[42] Damiano AE. Review: water channel proteins in the human placenta and fetal membranes. Placenta 2011;32(Suppl. 2):S207–11.