ARTICLE IN PRESS

Placenta xxx (2015) 1–3

Contents lists available at ScienceDirect

Placenta

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

Short communication

# Activity of $NA^+/H^+$ exchangers alters aquaporin-mediated water transport in human placenta

Valeria Dietrich [a], Alicia E. Damiano [a, b, *]

[a] Laboratorio de Biología de la Reproducción, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO), CONICET, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina
[b] Cátedra de Biología Celular y Molecular, Departamento de Ciencias Biológicas, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

*Article history:*
Received 30 May 2015
Received in revised form
17 September 2015
Accepted 30 September 2015

*Keywords:*
AQPs
NHEs
Water transport
pHi

ABSTRACT

The intracellular pH (pHi) of syncytiotrophoblasts is regulated, in part, by $Na^+/H^+$ exchanger (NHE)-1, NHE-2, and NHE-3. Failures in pHi homeostasis could alter critical cellular functions such as water transport and cell volume. Here, we evaluated whether alterations in syncytiotrophoblast pHi could modify water uptake mediated by aquaporins (AQPs) and the contribution of NHEs to this mechanism. We showed that changes in syncytiotrophoblast pHi did not affect water uptake in the presence of functional NHEs. However, inhibition of NHEs alters transcellular water transport mediated by AQPs in acidosis. These results suggest an interaction between placental AQPs and NHEs in the regulation of water uptake during acidotic states.

© 2015 Elsevier Ltd. All rights reserved.

## 1. Introduction

The primary function of the placenta is to promote selective transport of nutrients and waste products between the mother and fetus. This function depends on the successful formation and expansion of human villous syncytiotrophoblasts, which act as a physiological barrier between maternal and fetal blood. In addition, syncytiotrophoblast acid/base balance is necessary to maintain optimal solute transport rates across the placenta. However, the regulation of the intracellular pH (pHi) of syncytiotrophoblasts is not fully understood.

Three isoforms of the $Na^+/H^+$ exchanger (NHE) family, NHE-1, NHE-2, and NHE-3, and two of the $Cl^-/HCO_3^-$ anion exchangers (AEs), AE1 and AE2, have been identified in the human placenta and proposed to be involved in the maintenance of pHi of syncytiotrophoblasts [1–4].

In this regard, Illsley and coworkers suggested that cellular $Na^+$ and $H^+$ concentrations are closely controlled in syncytiotrophoblasts, and $Na^+/H^+$ antiporter primarily assisted syncytiotrophoblast cells in recovering from an intracellular acid load [5,6]. Moreover, studies by Cowley and coworkers showed that syncytial cells are protected from acidification solely by NHEs in the absence of $HCO_3^-$ [7].

Thus, failures in pHi homeostasis could alter various critical cellular functions such as water movements and cell volume regulation. Aquaporins (AQPs) are also key regulators of cell volume and intracellular ions. Five AQPs are expressed in the human placenta, although their functional significance remains unclear [8].

pHi was found to modify the selectivity of AQPs in other tissues [9–12].

Here, we hypothesized that changes in pHi by modifying NHE functionality may alter the selectivity of placental AQPs to water.

## 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 sample collection. Full-term normal placentas ($n = 6$) were obtained after delivery. All placentas were collected from white Hispanic pregnant women with no existing or previous history of disease who gave birth to a newborn without anomalies.

Fragments of cotyledons were gently separated by dissection from different areas of each placenta midway between the chorionic and basal plate. Villous tissue was further dissected into

* Corresponding author. Cátedra de Biología Celular y Molecular, 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).

http://dx.doi.org/10.1016/j.placenta.2015.09.014
0143-4004/© 2015 Elsevier Ltd. All rights reserved.

Please cite this article in press as: V. Dietrich, A.E. Damiano, Activity of $NA^+/H^+$ exchangers alters aquaporin-mediated water transport in human placenta, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.09.014

ARTICLE IN PRESS



explants of ~50 mg as previously described [13]. Cytosolic acidification was enforced by a $NH_4Cl$-pulse technique, as previously described [2,14]. Briefly, intracellular acidification was induced by an acid load ($NH_4Cl$ prepulse) to promote the activation of NHEs. The experiments were performed using Tyrode's buffer (in mM: NaCl 115, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, 3-(N-morpholino)propanesulfonic acid (MOPS) 10, and glucose 5.6), $NH_4Cl$-Tyrode's buffer (Tyrode's buffer with 20 mM $NH_4Cl$), and $Na^+$-free Tyrode's buffer (in mM: N-methyl-D-glucamine (DMG) 135, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1, MOPS 10, and glucose 5.6) at 37 °C and pH 7.4. The experimental protocol involved a 5-min $NH_4Cl$ prepulse, followed by a 3-min washout in $Na^+$-free Tyrode's buffer and a 5-min recovery phase in the presence of $Na^+$ (Tyrode's buffer). Amiloride, a nonselective inhibitor of NHEs (Sigma–Aldrich Co.; 0.5 mM); S3226, a selective inhibitor of NHE-3 [15] (Aventis Pharma, 0.1 mM); and 1% dimethyl sulfoxide (DMSO) (vehicle control) were dissolved in $Na^+$-free Tyrode's buffer and Tyrode's buffer to facilitate exposure of fragments during the $Na^+$-free washout period and during the recovery phase, respectively. Then, the fragments were incubated in 0.5 mL of hypo-osmolar saline solution containing radiolabeled water ($^3$HOH, New England Nuclear Co.; 1 mM) and water uptake was performed as previously described [16] using 0.3 mM $HgCl_2$ as a general blocker of AQPs. The uptake was stopped by adding ice-cold saline solution. Then, the fragments were quickly washed in cold saline solution, solubilized with 1 M NaOH, and kept overnight at 37 °C. Aliquots of the solubilized fragments were vortex-mixed with a scintillation liquid (Optiphase "HiSafe") and counted on a liquid scintillation counter. The aliquots were used to determine the protein concentration with a BCA Protein assay kit (Pierce). The uptake data ($pmol_{3HOH}$ $mg^{-1}{}_{protein}$ per minute) obtained from each group were compared by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test. The criterion for statistical significance was $P < 0.05$.

## 3. Results and discussion

As we expected, in the absence of the $NH_4Cl$ prepulse, water uptake did not change even in the presence of NHE inhibitors. After cytosolic acidification, water uptake remained unaltered when NHEs were not blocked. These results indicate that a disturbance of pH does not affect water movement in the presence of functional NHEs. However, we observed that water uptake markedly decreased when NHEs were inhibited (Fig. 1), which may occur when the physiological pH of the cells cannot be restored because of a failure in $H^+$ extrusion. Thus, inhibition of NHEs can lead to acidification of the cytoplasm, which may produce conformational changes in AQPs, in turn modifying their selectivity to water [17]. It should be noted that all experiments were performed in the absence of $HCO_3^-$ to avoid the contribution of placental AEs in the acid extrusion system, so only functional NHEs were allowed to minimize pHi changes. Moreover, no significant difference was found in the reduction of water uptake after inhibition by amiloride (which blocks all NHE isoforms) or S3226 (which blocks only NHE-3). These data suggest that NHE-3 might have a significant role during this process. As this isoform is proposed to contribute significantly to restoring pHi in trophoblast cells exposed to acid loads in vitro, we hypothesised that NHE-3 could be more sensitive to metabolic acidosis because of variations in placental nutrient or oxygen supply. Consequently, alterations in the expression and/or functionality of NHE-3 may severely affect the regulation of pHi in syncytiotrophoblast cells, impairing nutrient and/or water transport.

We also blocked AQPs to evaluate transcellular water transport. In previous experiments, in the absence of an acid load, we reported that placental water uptake was sensitive to $HgCl_2$. Here, we found that water uptake was also sensitive to $HgCl_2$ during cytosolic acidification, indicating the normal permeability of AQPs to water when functional NHEs are able to restore pHi (Fig. 2). However, in the presence of amiloride and S3226, water uptake was not only reduced but also insensitive to $HgCl_2$, suggesting that water does not pass through placental AQPs. Thus, the inhibition of the activity of NHEs may not only modify pHi homeostasis but also alter transcellular water transport mediated by AQPs.

In conclusion, our results show an interaction between AQPs and NHEs in the regulation of water uptake in the human placenta.

In this respect, we believe that the reduced expression of NHE-1 and NHE-3 [18,19] found in placentas of preeclamptic women may modify the pHi homeostasis and explain the lack of functionality of AQPs for water transport observed in these placentas [16]. Although

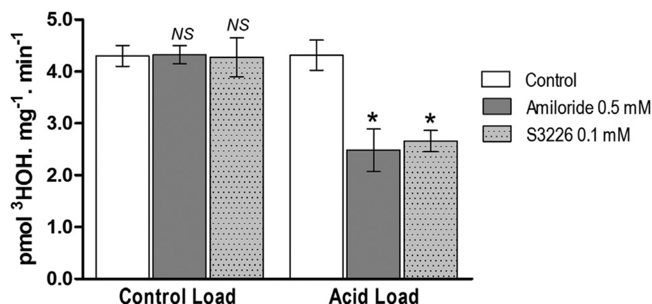


**Fig. 1.** Effect of cytosolic acidification on water uptake. Fragments of chorionic villi were exposed to intracellular acidification with a $NH_4Cl$ prepulse, and water uptake was evaluated in the presence of amiloride (nonselective NHE inhibitor) and S3226 (NHE-3-selective inhibitor). Under the control condition, the water uptake was 4.30 ± 0.21 pmol $mg^{-1}$ per minute. No significant change was observed when fragments were incubated with amiloride (4.33 ± 0.18 pmol $mg^{-1}$ per minute) or S3226 (4.28 ± 0.38 pmol $mg^{-1}$ per minute). When an acid load was imposed by $NH_4Cl$, water uptake showed no changes (4.32 ± 0.29 pmol $mg^{-1}$ per minute). However, when the fragments were incubated with amiloride or S3226, water uptake was significantly decreased (2.48 ± 0.41 and 2.66 ± 0.20 pmol $mg^{-1}$ per minute, respectively). The uptake data obtained from each assay performed in triplicate were compared by ANOVA followed by Fisher's LSD test ($n = 6$, $^*P < 0.05$ compared with control, *NS* = nonsignificant).

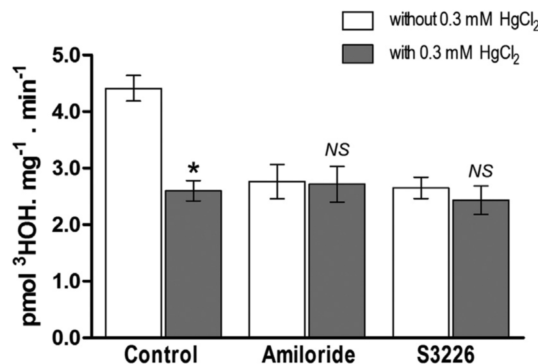


**Fig. 2.** Effect of cytosolic acidification and NHE inhibition on water uptake mediated by AQPs. After intracellular acidification with the $NH_4Cl$ prepulse, water uptake mediated by AQPs was evaluated in the presence of amiloride and S3226 in fragments of chorionic villi. As we expected, water uptake significantly decreased in the presence of $HgCl_2$ when NHEs were not inhibited (2.60 ± 0.18 vs. 4.41 ± 0.23 pmol $mg^{-1}$ per minute). However, when explants were incubated with amiloride or S3226, water uptake was significantly decreased (2.72 ± 0.31 and 2.43 ± 0.25 pmol $mg^{-1}$ per minute, respectively) and was not sensitive to $HgCl_2$ (2.76 ± 0.30 and 2.65 ± 0.19 pmol $mg^{-1}$ per minute, respectively). The uptake data obtained from each assay performed in triplicate were compared by ANOVA followed by Fisher's LSD test ($n = 6$, $^*P < 0.05$ compared to control without $HgCl_2$, *NS* = nonsignificant).

Please cite this article in press as: V. Dietrich, A.E. Damiano, Activity of $NA^+/H^+$ exchangers alters aquaporin-mediated water transport in human placenta, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.09.014

ARTICLE IN PRESS



the consequences of these alterations are not clear yet, they may contribute to the pathogenesis of gestational disorders such as preeclampsia.

## Funding

This study was supported by UBACyT 20020110200207 and PIP-CONICET 11220110100561 grants.

## Conflict of interest

The authors declare no conflict of interest that would prejudice the impartiality of this scientific work.

## Acknowledgments

The authors thank Dr. Bernardo Maskin, Dr. Mariana Jaime, and the staff of the Obstetric Unit of the Hospital Nacional “Dr. Prof. Alejandro Posadas”, Buenos Aires, Argentina, for their help in obtaining the placental tissues.

## References

[1] C.P. Sibley, J.D. Glazier, S.L. Greenwood, H. Lacey, K. Mynett, P. Speake, T. Jansson, M. Johansson, T.L. Powell, Regulation of placental transfer: the Na(+)/H(+) exchanger – a review, Placenta 23 (Suppl. A) (2002) S39–S46.
[2] P.F. Speake, K.J. Mynett, J.D. Glazier, S.L. Greenwood, C.P. Sibley, Activity and expression of Na+/H+ exchanger isoforms in the syncytiotrophoblast of the human placenta, Pflugers Arch. 450 (2) (2005) 123–130.
[3] S.M. Grassl, Cl/$HCO_3$ exchange in human placental brush border membrane vesicles, J. Biol. Chem. 264 (1989) 11103–11106.
[4] H.A. Lacey, T. Nolan, S.L. Greenwood, J.D. Glazier, C.P. Sibley, Gestational profile of Na+/H+ exchanger and Cl-/HCO3- anion exchanger mRNA expression in placenta using real-time QPCR, Placenta 26 (1) (2005) 93–98.
[5] N.P. Illsley, M.M. Jacobs, Control of the sodium-proton antiporter in human placental microvillous membranes by transport substrates, Biochim. Biophys. Acta 1029 (1990) 227–234.
[6] T.L. Powell, N.P. Illsley, A novel technique for studying cellular function in human placenta: gestational changes in intracellular pH regulation, Placenta 17 (8) (1996) 661–668.
[7] E.A. Cowley, M.C. Sellers, N.P. Illsley, Intracellular pH homeostasis in cultured human placental syncytiotrophoblast cells: recovery from acidification, Am. J. Physiol. Cell Physiol. 288 (4) (2005) C891–C898.
[8] A.E. Damiano, Review: water channel proteins in the human placenta and fetal membranes, Placenta 32 (Suppl. 2) (2011) S207–S211.
[9] H. Amlal, S. Sheriff, M. Soleimani, Upregulation of collecting duct aquaporin-2 by metabolic acidosis: role of vasopressin, Am. J. Physiol. Cell Physiol. 286 (5) (2004) C1019–C1030.
[10] T. Zeuthen, D.A. Klaerke, Transport of water and glycerol in aquaporin-3 is gated by H+, J. Biol. Chem. 274 (31) (1999) 21631–21636.
[11] T. Morishima, M. Aoyama, Y. Iida, N. Yamamoto, H. Hirate, H. Arima, Y. Fujita, H. Sasano, T. Tsuda, H. Katsuya, K. Asai, K. Sobue, Lactic acid increases aquaporin-4 expression on the cell membrane of cultured rat astrocytes, Neurosci. Res. 61 (1) (2008) 18–26.
[12] J.M. Carbrey, D.A. Gorelick-Feldman, D. Kozono, J. Praetorius, S. Nielsen, P. Agre, Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver, Proc. Natl. Acad. Sci. U. S. A. 100 (5) (2003) 2945–2950.
[13] M. Castro-Parodi, L. Levi, V. Dietrich, E. Zotta, A.E. Damiano, CFTR may modulate AQP9 functionality in preeclamptic placentas, Placenta 30 (7) (2009) 642–648.
[14] A. Roos, W.F. Boron, Intracellular pH, Physiol. Rev. 61 (2) (1981) 296–434.
[15] J.R. Schwark, H.W. Jansen, H.J. Lang, W. Krick, G. Burckhardt, M. Hropot, S3226, a novel inhibitor of $Na^+/H^+$ exchanger subtype 3 in various cell types, Pflügers Arch. 436 (5) (1998) 797–800.
[16] A.E. Damiano, E. Zotta, C. Ibarra, Functional and molecular expression of AQP9 channel and UT-A transporter in normal and preeclamptic human placentas, Placenta 27 (11–12) (2006) 1073–1081.
[17] A. Engel, Y. Fujiyoshi, P. Agre, The importance of aquaporin water channel protein structures, EMBO J. 19 (5) (2000) 800–806.
[18] I. Khan, M. Al-Yatama, M. Nandakumaran, Expression of the $Na^+$–$H^+$ exchanger isoform-1 and cyclooxigenases in human placentas: their implications in preeclampsia, Biochem. Mol. Biol. Int. 47 (4) (1999) 715–722.
[19] V. Dietrich, N. Szpilbarg, A.E. Damiano, Reduced expression of $Na^+/H^+$ exchanger isoform 3 (NHE-3) in preeclamptic placentas, Placenta 34 (9) (2013) 828–830.

Please cite this article in press as: V. Dietrich, A.E. Damiano, Activity of $NA^+/H^+$ exchangers alters aquaporin-mediated water transport in human placenta, Placenta (2015), http://dx.doi.org/10.1016/j.placenta.2015.09.014