Mistry, Hiten D. and Kurlak, L.O. and Whitley, Guy S. and Cartwright, Judith E. and Broughton Pipkin, Fiona and Tribe, Rachel M. (2014) Expression of voltage-dependent potassium channels in first trimester human placentae. Placenta, 35 (5). pp. 337-340. ISSN 1532-3102

Expression of voltage-dependent potassium channels in first trimester human placentae

Hiten D. Mistry[1,2*], Lesia O. Kurlak[3], Guy S. Whitley[4], Judith E. Cartwright [4], Fiona Broughton Pipkin[3] & Rachel M. Tribe[1]

[1]Division of Women's Health, King's College London, Women's Health Academic Centre, KHP, SE1 7EH, UK; [2]Department of Nephrology, Hypertension, Clinical Pharmacology and of Clinical Research, University of Bern, CH-3010 Berne, Switzerland; [3]Department of Obstetrics & Gynaecology, School of Medicine, University of Nottingham, NG5 1PB, UK; [4]Division of Biomedical Sciences, St George's University of London, SW17 0RE, UK.

***Correspondence address:** Dr. Hiten D. Mistry
Division of Women's Health
King's College London
Women's Health Academic Centre, KHP
St Thomas' Hospital
Westminster Bridge Road
London, SE1 7EH, UK
Tel: 020 71888151; Fax: 020 7620 1227
Email: hiten.mistry@kcl.ac.uk

Running Title: Placental K_V7 channels in early pregnancy

Keywords: Potassium channel, placenta, early pregnancy, KCNQ and KCNE.

Abstract

Potassium channel α-subunits encoded by KCNQ1-5 genes form voltage-dependent channels (Kv7), modulated by KCNE1-5 encoded accessory proteins. The aim was to determine KCNQ and KCNE mRNA expression and assess protein expression/localisation of the KCNQ3 and KCNE5 isoforms in first trimester placental tissue. Placentae were obtained from women undergoing elective surgical termination of pregnancy (TOP) at ≤10 weeks' (early TOP) and >10 weeks' (mid TOP) gestations. KCNQ1-5 expression was unchanged during the first trimester. KCNE5 expression increased in mid TOP vs. early TOP samples (P=0.022). This novel study reports mRNA and protein expression of Kv7 channels in first trimester placentae.

Introduction

Potassium (K^+) channel expression is essential for normal physiological functions of endothelial and smooth muscle cells in a variety of vascular beds [1]. Members of the Kv7 voltage-gated potassium channel subfamily Kv7.1-7.5 are encoded by KCNQ1-5 genes; the KCNQ-encoded α-subunits can form channel complexes with KCNE-encoded β-subunits (KCNE1-5). Alterations in K_V7 channel expression and their activation properties affect cell function, cell proliferation and differentiation [2].

Knowledge of K_V channels in the feto-placental circulation is limited [3, 4]. The presence of functional K_V7 channels in human chorionic plate arteries [5], suggests a role in control of vascular tone. Perfusion studies in placental allantochorial blood vessels incubated with K^+ channel blockers, exhibit responses to altered oxygenation, support this further [6-9].

We have previously reported raised Kv7 mRNA and protein expression in placental tissue from pre-eclamptic women compared to normotensive controls near term [3]. The reason for this difference is unknown; however these channels may be involved in early placentation, which is disrupted in pre-eclampsia. The aims of this study were to establish placental KCNQ/KCNE mRNA expression profiles in early pregnancy and to compare with previous observations from normotensive and pre-eclamptic women.

Materials and Methods

After local Ethical committee approval (Wandsworth Local Research Ethics Committee) and with appropriate informed consent, placental chorionic villous tissue, was obtained from women undergoing elective surgical TOP at St. George's Hospital, London during the early 1st trimester (early-TOP; ≤10 weeks', ([mean ± SD] 8.8 ± 0.9 weeks); n = 6) and late 1st trimester (mid-TOP, gestational age >10 weeks' (12.9 ± 0.9 weeks); n = 7). Samples were divided and either placed in RNA*later* (Qiagen, UK), stored at -80°C or fixed in formalin and wax embedded.

We compared the early pregnancy observations with data previously obtained using identical methodology, from placental tissue collected at delivery from 24 women with normotensive pregnancy (40.1 ± 1.2 weeks) and 22 women with pre-eclampsia (36.8 ± 3.6 weeks) [3].

Total RNA extraction, reverse transcription and real-time PCR were conducted as previously described [3]. Immunohistochemical staining was performed using goat polyclonal antibodies (KCNQ3 and KCNE5 (4 µg/ml for both), as previously described [3]. Goat IgG was used as a negative control. All slides were assessed by the same observer (HDM) and quantified using the Positive Pixel Algorithm of Aperio ImageScope software [3, 10].

Results

Expression of mRNA of KCNQ and KCNE genes was observed in first trimester tissues (Table 1). KNCQ4 and KCNQ5 were low or undetectable, whereas KCNQ3 and KCNQ1 showed the greatest expression. KCNE1, KCNE2 and KCNE4 expression was low in all TOP samples; KCNE5 was highly expressed isoform in mid-TOP and significantly greater than in early-TOP ($P=0.022$; Table 1).

High protein expression for both KCNQ3 and KCNE5 was observed, with staining being localised predominantly to the syncytiotrophoblast, cytotrophoblast and mesenchyme (Figure 1). No significant differences were observed between early and mid-TOP.

Lower mRNA expression of KCNQ2, KCNQ4, KCNQ5 and KCNE1 was observed in the TOP samples compared to the term placentae (Table 1). KCNQ3 and KCNE5 mRNA expression was significantly decreased in both early and mid-TOP and normotensive controls compared to pre-eclamptic placentae (Table 1).

Positive immunostaining for both KCNQ3 and KCNE5 was significantly lower in the normotensive and pre-eclamptic compared to both the early- and mid-TOP placentae (Table 1).

Discussion

This study presents novel data concerning placental KCNQ/KCNE mRNA expression profiles early in pregnancy. KCNQ3 and KNCE5 were the predominant isoforms, localised to syncytiotrophoblast and mesenchyme. This is similar to the profile in third trimester normotensive and pre-eclamptic placentae [3].

The only KCNQ isoforms to be significantly expressed in early pregnancy were KCNQ1 and KCNQ3. Both are known to be important for steroid production and inhibition of cell proliferation, essential factors in placentation [11-15]. KCNE expression was low in all early pregnancy, with the exception of KCNE5 in late-TOP placenta. In other studies, KCNE5 mRNA expression is markedly reduced in human 2^{nd} trimester trophoblast cells cultured under hypoxic conditions [16] and oxygen sensitive K^+ channels, including Kv channels, may be important for the detection and response to a oxygenation stimulus [7, 17] .

KCNQ3 and KCNE5 protein expression in early pregnancy was particularly high in the cytotrophoblast and syncytiotrophoblast. The presence of these proteins in the mesenchyme, the site of angiogenesis [18, 19] during this critical window of feto-placental vascular development, suggests a possible role for these proteins in vessel remodelling. Comparison with our third trimester normotensive and pre-eclamptic data indicate that KCNQ3 and KCNE5 proteins show similar localisation throughout pregnancy. KCNQ3 and KCNE5 expression was lower in tissues taken at delivery, but still raised in tissue from pre-eclampsia compared to normotensive controls. This lower expression, at term, could be due to changes in placental structure as pregnancy progresses, where reticulum cells and fibroblasts are the major cell types [20].

Contrasting data between mRNA and protein may be due to mRNA being less stable than protein and since such high expression of protein was observed in TOP samples, subtle differences may not be detected.

We had access to a limited number of samples at early gestations and unavoidably, the use of such samples precludes knowing whether these pregnancies may have developed pre-eclampsia. Nevertheless, taken together with our previous work on third trimester placentae, we have provided novel data suggesting a potential role for K_V7 channels in early placentation. Future work is needed to characterise the functional impact of KCNQ3 and KCNE5 co-expression both in the development of the early pregnancy placenta and in pre-eclampsia.

Acknowledgements

We thank all the patients who participated in the study and also to the nurses and doctors whose support in sample collection aided the successful completion of this project. We are also grateful to Dr. Laura McCallum for advice and suggestions during this study and to Mr Yosef Mansour for critical appraisal of the manuscript.

Grant Support: This work was supported by the Nottingham Hospitals Charity (LOK; Charity No. 1059049) and Tommy's Charity (HDM & RMT; Charity No. 1060508).

Conflict of Interest Statement: There are no conflicts of interest.

References:

[1] Wareing M. Oxygen sensitivity, potassium channels, and regulation of placental vascular tone. Microcirculation. 2014;21(1):58-66.

[2] Roura-Ferrer M, Sole L, Martinez-Marmol R, Villalonga N and Felipe A. Skeletal muscle Kv7 (KCNQ) channels in myoblast differentiation and proliferation. Biochemical and biophysical research communications. 2008;369(4):1094-7.

[3] Mistry HD, McCallum LA, Kurlak LO, Greenwood IA, Broughton Pipkin F and Tribe RM. Novel expression and regulation of voltage-dependent potassium channels in placentas from women with preeclampsia. Hypertension. 2011;58(3):497-504.

[4] Wareing M, Bai X, Seghier F, Turner CM, Greenwood SL, Baker PN, Taggart MJ and Fyfe GK. Expression and function of potassium channels in the human placental vasculature. American journal of physiology Regulatory, integrative and comparative physiology. 2006;291(2):R437-46.

[5] Mills TA, Shweikh Y, Greenwood SL, Jones RL and Wareing M. Control of human chorionic plate arterial tone: a role for Kv7 Channels? 2009. pp. 163A. Reproductive Sciences.

[6] Bisseling TM, Versteegen MG, van der Wal S, Copius Peereboom-Stegeman JJ, Borggreven JM, Steegers EA, van der Laak JA, Russel FG and Smits P. Impaired KATP channel function in the fetoplacental circulation of patients with type 1 diabetes mellitus. Am J Obstet Gynecol. 2005;192(3):973-9.

[7] Hampl V, Bibova J, Stranak Z, Wu X, Michelakis ED, Hashimoto K and Archer SL. Hypoxic fetoplacental vasoconstriction in humans is mediated by potassium channel inhibition. Am J Physiol Heart Circ Physiol. 2002;283(6):H2440-9.

[8] Guiet-Bara A and Bara M. Evidence of K and Ca channels in endothelial cells of human allantochorial placental vessels. Cell Mol Biol (Noisy-le-grand). 2002;48 Online Pub:OL317-22.

[9] Guiet-Bara A, Ibrahim B, Leveteau J and Bara M. Calcium channels, potassium channels and membrane potential of smooth muscle cells of human allantochorial placental vessels. Bioelectrochem Bioenerg. 1999;48(2):407-13.

[10] Williams PJ, Mistry HD, Innes BA, Bulmer JN and Broughton Pipkin F. Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human. Placenta. 2010;31(5):448-55.

[11] MacVinish LJ, Guo Y, Dixon AK, Murrell-Lagnado RD and Cuthbert AW. Xe991 reveals differences in K(+) channels regulating chloride secretion in murine airway and colonic epithelium. Molecular pharmacology. 2001;60(4):753-60.

[12] Greenwood IA, Yeung SY, Hettiarachi S, Andersson M and Baines DL. KCNQ-encoded channels regulate Na+ transport across H441 lung epithelial cells. Pflugers Archiv : European journal of physiology. 2009;457(4):785-94.

[13] Schroeder BC, Waldegger S, Fehr S, Bleich M, Warth R, Greger R and Jentsch TJ. A constitutively open potassium channel formed by KCNQ1 and KCNE3. Nature. 2000;403(6766):196-9.

[14] Demolombe S, Franco D, de Boer P, Kuperschmidt S, Roden D, Pereon Y, Jarry A, Moorman AF and Escande D. Differential expression of KvLQT1 and its regulator IsK in mouse epithelia. American journal of physiology Cell physiology. 2001;280(2):C359-72.

[15] Hamilton KL and Devor DC. Basolateral membrane K+ channels in renal epithelial cells. American journal of physiology Renal physiology. 2012;302(9):F1069-81.

[16] Luo Y, Kumar P and Mendelson CR. Estrogen-Related Receptor gamma (ERRgamma) Regulates Oxygen-Dependent Expression of Voltage-gated Potassium (K+) Channels and Tissue Kallikrein during Human Trophoblast Differentiation. Mol Endocrinol. 2013;27(6):940-52.

[17] Archer S and Michelakis E. The mechanism(s) of hypoxic pulmonary vasoconstriction: potassium channels, redox O(2) sensors, and controversies. News Physiol Sci. 2002;17:131-7.

[18] Fatimah SS, Tan GC, Chua K, Fariha MM, Tan AE and Hayati AR. Stemness and angiogenic gene expression changes of serial-passage human amnion mesenchymal cells. Microvascular research. 2013;86:21-9.

[19] Kohn C, Dubrovska G, Huang Y and Gollasch M. Hydrogen sulfide: potent regulator of vascular tone and stimulator of angiogenesis. International journal of biomedical science : IJBS. 2012;8(2):81-6.

[20] Kaufmann P, Mayhew TM and Charnock-Jones DS. Aspects of human fetoplacental vasculogenesis and angiogenesis. II. Changes during normal pregnancy. Placenta. 2004;25(2-3):114-26.

Figure Legends

Figure 1: A) KCNQ3 B) KCNE5 immunostaining in 1) early-TOP, 2) mid-TOP and 3) IgG negative controls. In photomicrographs, positive cells appear brown; magnification x400. High protein expression and was localised mainly to the syncytiotrophoblast (red arrows), but was also evident in the mesenchyme (blue arrows). In graphs, data are presented as median [IQR] positivity; scale bar = 100 µm.

229 **Table 1:** KCNQ and KCNE mRNA and protein expression isoform placental.

mRNA expression (normalised GAPDH, copy number) x100[#]	**Early TOP (n = 6)**	**Mid TOP (n = 7)**	**Normotensive Control (n = 24)**	**Pre-eclampsia (n = 22)**
KCNQ1	0.4 [0.3, 0.5]	0.3 [0.3, 0.5]	3.54 [2.0, 10.4]	5.6 [2.0, 11.0]
KCNQ2	0.2 [0.1, 0.4][e]	0[b]	0.7 [0.2, 1.6]	1 [0.3, 2]
KCNQ3	0.3 [0.2, 0.4][e]	0.4 [0.2, 0.8][f]	2.3 [0.8, 6.8][c]	46,967 [26,457, 10,2570]
KCNQ4	0 [0, 0.03][a]	0	0.4 [0.2, 0.7]	0.2 [0.1, 0.6]
KCNQ5	0.01 [0, 0.02][a]	0.04 [0, 0.08][b]	8.3 [2.9, 20.3][c]	2.2 [0.6, 7.2]
KCNE1	0.02 [0, 0.02][a]	0.04 [0, 0.08][b]	1.5 [1, 4.3]	0.8 [0.5, 2.2]
KCNE2	0.07 [0.03, 0.08]	0.09 [0.06, 0.1]	0.04 [0, 0.08]	0.02 [0, 0.1]
KCNE3	0.9 [0.6, 1.0]	0.7 [0.6, 1]	1.5 [0.8, 3.2]	1.2 [0.7, 3]
KCNE4	0.2 [0.2, 0.5]	0.2 [0.2, 0.3]	0.2 [0.1, 0.5]	0.3 [0.2, 0.6]
KCNE5	0.6 [0.4, 1][a]	2 [1, 3][g]	16 [9, 29][c]	194 [91, 338]
Positive immunostaining (arbitrary units)[#]	**Early TOP (n = 7)**	**Mid TOP (n = 5)**	**Normotensive Control (n = 6)**	**Pre-eclampsia (n = 6)**
KCNQ3	0.97 [0.96, 0.99][a]	0.99 [0.96, 0.99][b]	0.18 [0.1, 0.2][c]	0.31 [0.2, 0.35]
KCNE5	0.98 [0.94, 0.99][a]	0.99 [0.98, 0.99][b]	0.13 [0.06, 0.16][c]	0.35 [0.28, 0.43]

230

231 [a]: P<0.05 early TOP vs. normotensive controls and pre-eclampsia; [b]: P<0.05 mid TOP vs.

232 normotensive controls and pre-eclampsia; [c]: P<0.05 normotensive controls vs. pre-eclampsia; [d]:

233 P<0.05 early TOP vs. normotensive controls; [e]: P<0.05 early TOP vs. pre-eclampsia; [f]: P<0.05

234 mid TOP vs. normotensive controls; [g]: P<0.05 mid TOP vs. pre-eclampsia. Data presented as

235 median [IQR]. [#]Normotensive control and pre-eclampsia data previously published [3].