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Placental expression of adenosine A_{2A} receptor and hypoxia inducible factor-1 alpha in early pregnancy, term and pre-eclamptic pregnancies; interactions with placental renin-angiotensin system

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Running Title: Placental $A_{2A}R$, HIF-1α and RAS receptors in early pregnancy and pre-eclampsia.

Abstract

Normal placentation occurs under low oxygen tensions yet hypoxia is also implicated in placental pathologies such as pre-eclampsia (PE). Hypoxia-inducible factors (HIFs), adenosine and tissue renin-angiotensin-system (RAS) are known to promote angiogenesis and vascularisation. We hypothesised that placental adenosine $A_{2A}R$ receptor and HIF-1α would change through pregnancy in association with the RAS. Placentae were obtained from women undergoing elective surgical termination of pregnancy (TOP) at ≤10 weeks' (early TOP) and >10 weeks' (mid TOP) gestations; at delivery from normotensive (NT) and PE pregnancy. Results were compared to our previously reported data on the angiotensin receptors: AT1R, AT2R and AT4R.

Protein expression of both $A_{2A}R$ and HIF-1α was highest in early TOP and positively correlated through pregnancy ($P<0.0001$): expression was higher in PE than NT at delivery ($P<0.0001$ for both). The $A_{2A}R$ positively correlated with the AT4R in placentae in early pregnancy ($r=0.53$; $P=0.035$), but not in 3rd trimester samples. Our findings suggest a role for adenosine and RAS in promoting placentation and as a potential adaptation to poor placental perfusion in pre-eclampsia.

Introduction

Adenosine modulates a number of physiological processes including promotion of angiogenesis, cell proliferation, inflammation, regulation of vascular tone and protection against oxidative stress [1]: hypoxia stimulates release of adenosine [2]. Normal placentation develops under low oxygen conditions [3] with oxygen sensing hypoxia-inducible factors (HIFs) regulating the transcription of genes that participate in angiogenesis and invasion enhancing vascularization.

Both angiotensin peptides, AngII and AngIV, acting through receptors AT2R and AT4R, have similar effects to adenosine in promoting angiogenesis, proliferation, inflammation and protection against oxidative stress [4]. In early pregnancy both AT2R and AT4R are highly expressed, contributing to normal placental development [5] including extravillious trophoblast invasion [6].

In PE, where impaired placentation leads to an inadequate feto-placental circulation and increased oxidative stress, maternal and umbilical venous adenosine [7] and placental HIF-1α concentrations [8, 9] are raised as is placental AT1R expression [6] whilst conversely, AT4R expression is reduced [6].

The aims of this study were to 1) demonstrate the presence and distribution of the $A_{2A}R$ and HIF-1α in early TOP (8-10 weeks gestational age) when placental oxygen tension is low,mid (12-14 weeks gestational age) after spiral artery blood flow is established and at term in NT and PE placentae. 2) To relate these to the angiotensin receptors known to be involved in placentation.

Methods

After local Ethical Committee approval and with appropriate informed consent, placental tissue identified by its macroscopic appearance, was dissected from products of conception obtained from women undergoing elective surgical termination of pregnancy (TOP) at 8-10 weeks (early TOP; n = 8; gestational age 8.8 ± 0.9 weeks [Mean ± S.D.]) and 12-14 weeks' gestational age (mid TOP; n = 11; gestational age 12.7 ± 1.0 weeks). Placental samples were also taken at delivery in the third trimester from 11 women with normotensive pregnancy (39.1 ± 1.4 weeks) and 10 women with PE (37.6 ± 2.6 weeks) who underwent Caesarean section prior to labour. We compared these observations with angiotensin receptor data previously obtained in these samples using identical methodology [6].

Immunohistochemical staining was performed using rabbit polyclonal antibodies ($A_{2A}R$ and HIF-1α (10 μg/ml), as previously described [6]. Rabbit IgG was used as negative controls. All slides were assessed by the same observer and quantified using the Positive Pixel Algorithm of Aperio ImageScope software [6, 10].

Results

Both $A_{2A}R$ (Fig.1A) and HIF-1α (Fig. 1B) proteins were confined to villous syncytiotrophoblasts and cytotrophoblasts with immunochemical intensity being stronger in the syncytiotrophoblast outer layer of the villi than in the underlying cytotrophoblast layer. Expression of both proteins was highest at 8-10 weeks gestation, decreasing at 12-14 weeks, and falling further to term in normotensive placentae ($P<0.0001$ for all; Fig. 1). However, for both proteins, placental expression was similar in placentae from mid-TOP and PE $P>0.05$). Moreover, both proteins were higher in PE compared to controls ($P<0.001$). A very strong positive correlation was found between $A_{2A}R$ and HIF-1α in all groups overall throughout pregnancy ($r = 0.85$; $P < 0.0001$).

The protein expression of the RAS receptors has previously been reported [6]. Positive correlations were observed between $A_{2A}R$ and both AT2R ($r = 0.52$; $P=0.033$) and AT4R ($r = 0.55$; $P=0.023$) in placentae obtained before 20 weeks' gestation. However, AT2R and AT4R expression were themselves strongly correlated at this gestational age ($r = 0.485$; $P<0.002$) and partial correlation analysis showed $A_{2A}R$ to be significantly correlated only with AT4R expression ($r = 0.53$; $P=0.035$; Fig. 2). No such correlation was found for the term normotensive or pre-eclamptic pregnancies. Placental expression of HIF-1α was independent of RAS receptor expression at all gestations.

Discussion

This study demonstrates the expression and cellular localisation of both $A_{2A}R$ and HIF-1α spanning first trimester to term. We uniquely report a strong correlation between these proteins over this period. The higher expression of $A_{2A}R$ and HIF-1α in PE, despite similar gestational ages to the NT pregnancies, suggests that this association is not purely a developmental phenomenon, but related to tissue oxygen status. The correlation between $A_{2A}R$ with the angiotensin receptor - AT4R raises the possibility of a novel interaction of adenosine and the RAS during early placentation. AT4R has already been implicated in the regulation of blood flow [11], being present in both endothelial [12] and smooth muscle cells [13]. Moreover, chronic hypoxia upregulates AT4R in the carotid body [14], with an increase in AngIV-induced intracellular [Ca^{2+}]. Interestingly, adenosine also raises intracellular [Ca^{2+}] via cyclic AMP under these conditions. Of note, activation of AT4R can produce endogenous vasodilatory nitric oxide via activation of endothelial nitric oxide synthase (eNOS) [15] and likewise, adenosine increases nitric oxide synthesis from feto-placental endothelium [16, 17]. The loss of the correlation between $A_{2A}R$ and AT4R in the term samples, whether NT or PE, is suggestive of a specific interaction during placental growth.

There are a number of possible mechanisms to explore and future work is required to fully characterise the functional impact of $A_{2A}R$, AT2R and AT4R both in early placental development and in relation to PE.

References

1. Hasko, G. and B.N. Cronstein, *Adenosine: an endogenous regulator of innate immunity.* Trends Immunol, 2004. **25**(1): p. 33-9.
2. Poth, J.M., et al., *Transcriptional control of adenosine signaling by hypoxia-inducible transcription factors during ischemic or inflammatory disease.* J Mol Med (Berl), 2013. **91**(2): p. 183-93.
3. Rodesch, F., et al., *Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy.* Obstet Gynecol, 1992. **80**(2): p. 283-5.
4. Broughton Pipkin, F., *The renin-angiotensin system in pre-eclampsia*, in *Pre-eclampsia*, F. Lyall and M. Belfort, Editors. 2007, Cambridge University Press: Cambridge. p. 78-91.
5. Tebbs, C., M.K. Pratten, and F. Broughton Pipkin, *Angiotensin II is a growth factor in the peri-implantation rat embryo.* J Anat, 1999. **195 (Pt 1)**: p. 75-86.
6. Williams, P.J., et al., *Expression of AT1R, AT2R and AT4R and their roles in extravillous trophoblast invasion in the human.* Placenta, 2010. **31**(5): p. 448-55.
7. Yoneyama, Y., et al., *Increased plasma adenosine concentrations and the severity of preeclampsia.* Obstet Gynecol, 2002. **100**(6): p. 1266-70.
8. Caniggia, I. and J.L. Winter, *Adriana and Luisa Castellucci Award lecture 2001. Hypoxia inducible factor-1: oxygen regulation of trophoblast differentiation in normal and pre-eclamptic pregnancies--a review.* Placenta, 2002. **23 Suppl A**: p. S47-57.
9. Rajakumar, A., et al., *Proteasomal activity in placentas from women with preeclampsia and intrauterine growth restriction: implications for expression of HIF-alpha proteins.* Placenta, 2008. **29**(3): p. 290-9.

10. Mistry, H.D., et al., *Novel expression and regulation of voltage-dependent potassium channels in placentas from women with preeclampsia.* Hypertension, 2011. **58**(3): p. 497-504.

11. Kramar, E.A., J.W. Harding, and J.W. Wright, *Angiotensin II- and IV-induced changes in cerebral blood flow. Roles of AT1, AT2, and AT4 receptor subtypes.* Regul Pept, 1997. **68**(2): p. 131-8.

12. Hall, K.L., et al., *Characterization of a functional angiotensin IV receptor on coronary microvascular endothelial cells.* Regul Pept, 1995. **58**(3): p. 107-15.

13. Hall, K.L., et al., *Identification and characterization of a novel angiotensin binding site in cultured vascular smooth muscle cells that is specific for the hexapeptide (3-8) fragment of angiotensin II, angiotensin IV.* Regul Pept, 1993. **44**(2): p. 225-32.

14. Fung, M.L., et al., *Carotid Body AT(4) Receptor Expression and its Upregulation in Chronic Hypoxia.* Open Cardiovasc Med J, 2007. **1**: p. 1-7.

15. Patel, J.M., et al., *Angiotensin IV receptor-mediated activation of lung endothelial NOS is associated with vasorelaxation.* Am J Physiol, 1998. **275**(6 Pt 1): p. L1061-8.

16. Casanello, P., C. Escudero, and L. Sobrevia, *Equilibrative nucleoside (ENTs) and cationic amino acid (CATs) transporters: implications in foetal endothelial dysfunction in human pregnancy diseases.* Curr Vasc Pharmacol, 2007. **5**(1): p. 69-84.

17. Escudero, C. and L. Sobrevia, *A hypothesis for preeclampsia: adenosine and inducible nitric oxide synthase in human placental microvascular endothelium.* Placenta, 2008. **29**(6): p. 469-83.

Figure Legends

Figure 1: Immunohistochemical quantification and localization of A) $A_{2A}R$ and B) HIF-1α at 8-10 weeks gestation (A1 & B1), 12-14 weeks gestation (A2 & B2), term normotensive control (A3 & B3) and PE placentae (A4 & B4). (A5 & B5) demonstrates the negative control. Data are presented as median [IQR]; ***$P < 0.0001$. All photomicrographs taken at x200 magnification; positive staining is shown in brown, black arrows indicate villous cytotrophoblast cells and red arrows show villous syncytiotrophoblast cells.

Figure 2: Scatter plot illustrating the positive association between $A_{2A}R$ and AT4R in placentae < 20 weeks gestation ($r = 0.53$; $P = 0.035$).