Placenta 34 (2013) 1121–1127

Contents lists available at ScienceDirect

Placenta

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

Current topic

Role of equilibrative adenosine transporters and adenosine receptors as modulators of the human placental endothelium in gestational diabetes mellitus

F. Pardo [a,*], P. Arroyo [a], C. Salomón [a,b], F. Westermeier [a], R. Salsoso [a], T. Sáez [a], E. Guzmán-Gutiérrez [a], A. Leiva [a], L. Sobrevia [a,b,*]

[a] Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile
[b] The University of Queensland Centre for Clinical Research, Faculty of Health Sciences, The University of Queensland, Herston, QLD, Australia

ARTICLE INFO

Article history:
Accepted 13 September 2013

Keywords:
Gestational diabetes
Placenta
Endothelium
Adenosine
Insulin

ABSTRACT

Gestational diabetes mellitus (GDM) is a diseases that alters human placenta macro and microvascular reactivity as a result of endothelial dysfunction. The human placenta is a highly vascularized organ which lacks innervation, so blood flux is governed by locally released vasoactive molecules, including the endogenous nucleoside adenosine and the free radical nitric oxide (NO). Altered adenosine metabolism and uptake by the endothelium leads to increased NO synthesis which then turns-off the expression of genes coding for a family of nucleoside membrane transporters belonging to equilibrative nucleoside transporters, particularly isoforms 1 (hENT1) and 2 (hENT2). This mechanism leads to increased extracellular adenosine and, as a consequence, activation of adenosine receptors to further sustain a tonic activation of NO synthesis. This is a phenomenon that seems operative in the placental macro and microvascular endothelium in GDM. We here summarize the findings available in the literature regarding these mechanisms in the human feto-placental circulation. This phenomenon is altered in the feto-placental vasculature, which could be crucial for understanding GDM deleterious effects in fetal growth and development.

1. Introduction

Gestational diabetes mellitus (GDM) is a disease characterized by glucose intolerance leading to maternal hyperglycaemia with onset or first recognition during pregnancy, whose incidence accounts for 5–15% of pregnancies in developed and developing countries [1]. One of the consequences of GMD is an altered vascular function [2,3], documented to result in changes at the prenatal life with severe perinatal alterations (e.g., macrosomia [4], insulin resistance [5], higher systolic blood pressure [6]), and diseases in the adulthood (e.g., diabetes [7], obesity [8], dyslipidaemia [9], hypertension [9] and metabolic syndrome [10]). Since the placenta lacks innervation [11], locally released vasoactive molecules from the endothelium, such as the gas nitric oxide (NO) or the endogenous nucleoside adenosine [12], play key roles in maintaining a physiological placenta vascular function [12–15].

Altered function of the placental endothelium (i.e., endothelial dysfunction) is defined as an altered capacity of the endothelium to take up and metabolize the cationic amino acid L-arginine, the substrate for NO synthesis via NO synthases (NOS) [13]. GDM is associated with altered transport of L-arginine and NO synthesis (i.e., the 'L-arginine/NO signalling pathway') [2,3,15]. Adenosine is a vasodilator in most vascular beds [14,16–18], including the human placenta [19,20]. The latter results from increased L-arginine transport-dependent NO synthesis via the endothelial NO synthase (eNOS). Thus, a functional link between adenosine and endothelial L-arginine/NO pathway has been proposed [21], This phenomenon associates with lower capacity of adenosine transport via human equilibrative nucleoside transporters (hENTs) by human umbilical vein endothelial cells (HUVEC) and placental microvascular endothelial cells (hPMEC) in GDM. Thus, extracellular accumulation of this nucleoside facilitates activation of endothelial adenosine receptors. The latter was proposed as a mechanism to explain

* Corresponding authors. Cellular and Molecular Physiology Laboratory (CMPL), Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, P.O. Box 114-D, Santiago, Chile. Tel.: +562 23548117; fax: +562 26321924.
E-mail addresses: fpardo@med.puc.cl (F. Pardo), sobrevia@med.puc.cl (L. Sobrevia).

0143-4004/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.placenta.2013.09.007

endothelial dysfunction in hyperglycaemia and in hyperglycaemia-associated pregnancy diseases such as GDM [3,12]. We here summarize findings supporting the possibility that GDM is a disease that alters the fetoplacental endothelial function due to a deficient handling of adenosine with consequences on adenosine receptors activation and modulation of placental vascular function.

2. Adenosine

Adenosine is an endogenous purine nucleoside continuously formed in the intra and the extracellular media. Intracellular synthesis of this nucleoside is mediated by 5′-nucleotidases, which dephosphorylates AMP, or by hydrolysis of *S*-adenosyl-homocysteine [16,22]. Production of adenosine at the extracellular space derives from dephosphorylation of extracellular AMP via ecto-5′-nucleotidases present at the endothelial cells plasma membrane [23], including HUVEC [16]. Once adenosine is taken up into the endothelium, it gets phosphorylated by adenosine kinases to form AMP or degraded by adenosine deaminase into inosine [22]. Adenosine triggers numerous physiological responses via activation of adenosine receptors, which are members of the G protein-coupled receptors superfamily widely distributed throughout different tissues and cell types [14,17,22]. The main biological effects of adenosine are to maintain the homeostatic equilibrium depending on the type of adenosine receptors involved (Table 1). These biological actions of adenosine include modulation of energy homeostasis (ATP metabolism), acting as a trigger of cell signal transduction mechanisms mediated by cAMP [17,22], and activation of the endothelial L-arginine/NO signalling pathway [2,3,17,24]. Additionally, adenosine plays key roles as anti-inflammatory [18,25] and as key stimulator of angiogenesis [18].

2.1. Adenosine receptors and placental endothelium

Four subtypes of adenosine receptors belonging to the P_1 family of membrane receptors have been identified, i.e., A_1 adenosine receptors (A_1AR), A_{2A}AR, A_{2B}AR and A_3AR [17,22]. Adenosine receptor subtypes exhibit a different order of potency of ligand binding and cell signal [22]. A_1AR and A_3AR activation leads to inhibition of adenylyl cyclase and activation of phosphatidylinositol 3-kinase (PI3k)/Akt pathway, while A_{2A}AR and A_{2B}AR activates adenylyl cyclase [14,17,22]. All adenosine receptor isoforms are expressed in the human placental endothelium [26,27] and trophoblast cells [28,29], but only A_1AR,, A_{2A}AR and A_3AR in placental fibroblasts [28]. Thus, adenosine receptors expression in different tissues is crucial regarding differential adenosine biological effects [14,17,22].

Adenosine also plays different roles in vascular tissues, including regulation of vascular tone and the blood flow [14,17,18,30]. To date, A_1AR activation by low partial pressure of oxygen has been shown to increase intracellular concentration of calcium (Ca^{2+}) leading to higher NO synthases (NOS) activity and NO synthesis, while A_{2A}AR activation induces NO-dependent vasodilation in rat aortic endothelial cells [31]. Other reports show abundant A_{2A}AR, A_{2B}AR and A_3AR, but relative low A_1AR expression in HUVEC primary cultures [32,33], and only A_{2A}AR and A_{2B}AR has been reported in hPMEC [26]. Thus, a differential expression of adenosine receptor subtypes is a factor contributing to the functional heterogeneity of human placental macro and microvascular endothelium [12].

2.2. Nucleoside transporters and placental endothelium

There are two families of nucleoside transporters that have been identified in mammalian cells, i.e., Na^+-independent equilibrative nucleoside transporters (ENTs) and Na^+-dependent concentrative nucleoside transporters (CNTs) [34,30]. Four members of ENTs family of solute carriers (*SLC29A* gene) have been cloned, i.e., hENT1, hENT2, hENT3 and hENT4 (Table 2). hENT1 and hENT2 are key membrane transporters in the vasculature, hENT3 is a lysosomal transporter, while hENT4, also described in HUVEC, requires an extracellular pH ~5.5 to be active [34–36]. hENT1 is a protein of

Table 1
Adenosine receptor subtypes and main cardiovascular physiological effects.

Receptor	Cell signalling	Cellular localization	Physiological function	Reference
A_1	Low cAMP	Heart	Regulation of Ca^{2+} and K^+ channels	[65]
			Protection against ischemia	[66]
		Endothelial cells	Synthesis and release of NO	[47,66]
			Vasodilation	[47,66]
		Macrophages	Macrophage activation	[67]
			Release of cytokines	[67]
		Leucocytes	Promotion of adhesion to endothelial cells	[68]
			Release of proinflammatory mediators	[68]
A_{2A}	High cAMP	Endothelial cells	Synthesis and release of NO	[31,69]
			Endothelium-dependent vasodilation	[31,69]
			Required for insulin biological effect	[56]
		Macrophages	Macrophage activation	[70]
			Release of cytokines	[70]
		Leucocytes	Promotion of adhesion to endothelial cells	[71]
			Release of proinflammatory mediators	[71]
		Lymphocytes	Regulation of proliferation	[72]
A_{2B}	High cAMP	Endothelial cells	Synthesis and release of NO	[69]
			Vasodilation	[69]
		Macrophages	Macrophage activation	[73]
			Release of cytokines	[74]
		Leucocytes	Promotion of adhesion to endothelial cells	[74]
			Release of proinflammatory mediators	[75]
		Lymphocytes	Lymphocyte activation	
A_3	Low cAMP	Heart	Protection against ischemia	[66,76]
		Macrophages	Macrophage activation	[77]
			Release of cytokines	[77]
		Leucocytes	Promotion of adhesion to endothelial cells	[78]
			Release of proinflammatory mediators	[78]

cAMP, cyclic adenosine monophosphate; NO, nitric oxide.