Placenta 34 (2013) 953–958

Contents lists available at SciVerse ScienceDirect

Placenta

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

What factors determine placental glucose transfer kinetics?☆

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ARTICLE INFO

Article history:
Accepted 5 July 2013

Keywords:
Placenta
Glucose
Membrane transport
Kinetics

ABSTRACT

Introduction: Transfer of glucose across the human placenta is directly proportional to maternal glucose concentrations even when these are well above the physiological range. This study investigates the relationship between maternal and fetal glucose concentrations and transfer across the placenta.
Methods: Transfer of D-glucose, ^{3}H-3-o-methyl-D-glucose (^{3}H-3MG) and ^{14}C-L-glucose across the isolated perfused human placental cotyledon was determined for maternal and fetal arterial D-glucose concentrations between 0 and 20 mmol/l.
Results: Clearance of ^{3}H-3MG or ^{14}C-L-glucose was not affected by maternal or fetal D-glucose concentrations in either circulation.
Discussion: Based on the arterial glucose concentrations and the reported K_M for GLUT1, the transfer of D-glucose and ^{3}H-3MG would be expected to show signs of saturation as D-glucose concentrations increased but this did not occur. One explanation for this is that incomplete mixing of maternal blood and the rate of diffusion across unstirred layers may lower the effective concentration of glucose at the microvillous membrane and subsequently at the basal membrane. Uncertainties about the affinity of GLUT1 for glucose, both outside and inside the cell, may also contribute to the difference between the predicted and observed kinetics.
Conclusion: These factors may therefore help explain why the observed and predicted kinetics differ and they emphasise the importance of understanding the function of transport proteins in their physiological context. The development of a computational model of glucose transfer may improve our understanding of how the determinants of placental glucose transfer interact and function as a system.

1. Introduction

Placental glucose transfer is essential to sustain fetal growth and metabolism. Insufficient glucose transfer will result in fetal growth restriction (FGR) while too much is associated with fetal macrosomia [1]. FGR and macrosomia are associated with complications at birth and an increased burden of chronic diseases in adulthood [2]. As such, it is important to understand factors that determine glucose availability to the fetus in pregnancies with normal and abnormal maternal glucose levels.

Glucose transport across the microvillous (MVM) and basal (BM) membranes of the human placental syncytiotrophoblast at term has been shown to be a transporter mediated process [3]. It is thought that glucose transfer is predominantly mediated by the transporter GLUT1 (SLC2A1) which mediates facilitated diffusion [3,4]. GLUT1 levels are lower on the BM which also has a smaller surface area and, as a result, is thought to be rate-limiting for placental glucose transfer [4]. Glucose transporters other than GLUT1 may play important roles earlier in gestation, especially GLUT3 [5,6]. The consensus K_M for D-glucose transport by GLUT1 in human erythrocytes is reported to be around 3 mmol/l although lower and higher K_M values have been reported, ranging from 1 to 17 mmol/l [7,8]. Glucose uptake by human placental MVM and BM membrane vesicles is very rapid suggesting a high capacity for glucose transport in the placenta [9]. Net glucose transfer across the placenta will be the sum of paracellular and transcellular routes, less any metabolised in the placenta. Although the paracellular route across the syncytiotrophoblast is poorly defined, it has been demonstrated using L-glucose which is not transported by glucose transporters [8,10,11].

The rate of glucose transfer across the human placenta is thought to be primarily determined by the maternal to fetal glucose gradient and transfer is directly proportional to this gradient up to maternal glucose concentrations well above physiological [10,12,13]. Evidence of transporter saturation in the perfused placenta has been reported with glucose concentrations above 20 mmol/l suggesting

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http://dx.doi.org/10.1016/j.placenta.2013.07.001

an apparent K_M for placental glucose transport higher than that reported for GLUT1 [3]. Maternal and fetal blood flow will maintain the concentration gradient by delivering glucose enriched blood to the maternal side and removing the glucose transported to the fetal circulation. Evidence that glucose transfer is flow-limited is consistent with the idea that glucose transfer increases linearly with maternal–fetal glucose gradients [14]. This is interesting as a high affinity transporter such as GLUT1, which is believed to have a K_M below physiological glucose concentrations, would be expected to be increasingly independent of maternal glucose concentrations as they rise above the K_M as illustrated in Fig. 1A. If there are more transporters in the membrane then V_{max} will be higher and the capacity for glucose transfer across the placenta will be greater. However, increasing V_{max} will not change the glucose concentration at which transporter saturation will occur (Fig. 1B).

Given the difference between the observed glucose transfer in the human placenta and what would be predicted based on the kinetics of GLUT1, we explored the extent to which glucose transfer is transporter-limited. We also addressed the role of paracellular glucose transfer in the human placenta.

2. Methods

Human placentas from uncomplicated term deliveries were obtained immediately after delivery from Princess Anne Maternity Hospital. Ethical approval was given by the South and West Hants Local Research Ethical Committee.

3. Placental perfusion methodology

Placentas were perfused using the methodology of Schneider [15] as adapted in our laboratory [16]. All placentas used for the studies had a fetal arterial flow rate recovery of 95% or greater. Earle's Bicarbonate Buffer (EBB, 1.8 mM $CaCl_2$, 0.4 mM $MgSO_4$, 116.4 mM NaCl, 5.4 mM KCl, 26.2 mM $NaHCO_3$, 0.9 mM NaH_2PO_4 and a variable concentration of glucose as stated below) gassed with 5% CO_2 and 95% O_2 was perfused through the fetal catheter going into the chorionic plate fetal artery at 6 ml/min and the five maternal catheters at 14 ml/min using a roller pump. Buffers containing D-glucose, ^{14}C-L-glucose and ^{3}H-3-o-methyl-D-glucose (^{3}H-3MG) (Perkin Elmer, Massachusetts, USA), were then perfused as described below. When sampling approximately 1.5 ml of venous exudate was collected from maternal and fetal venous outflows. At the end of the experiments, the perfused mass of placental cotyledon that assumed a white colour was obtained by trimming off the non-perfused tissue, then blotted and weighed.

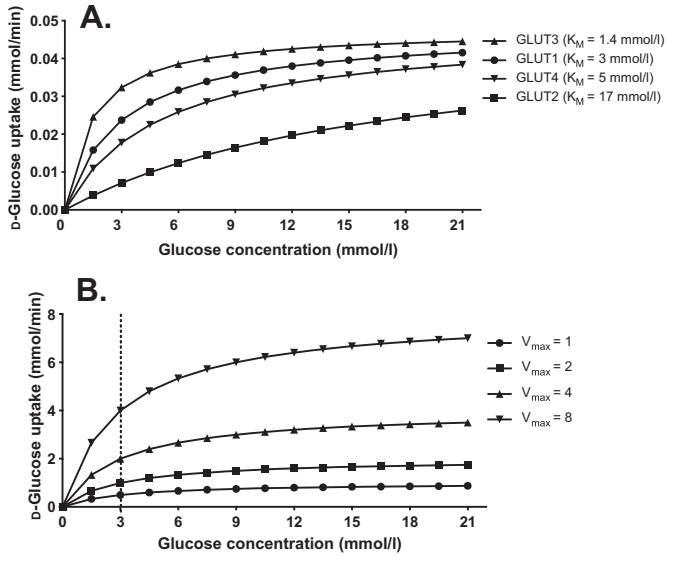

Fig. 1. The predicted effect of K_M and V_{max} on glucose transport based on simple Michaelis Menten Kinetics. A, The effect of K_M on glucose uptake using the K_Ms for GLUT1-4 as examples [7]. Glucose transfer by GLUTs with a lower K_M would be less dependent on maternal glucose concentration. B, Glucose uptake is directly proportional to V_{max} but only proportional to maternal glucose levels where these are less than K_M. Note that the K_M for all curves is 3 mmol/l (dotted line) regardless of the V_{max}.

4. Glucose perfusions

In initial experiments increasing concentrations of D-glucose were perfused through the maternal artery (from 0 to 18 mmol/l in 3 mmol/l steps for 20 min each) with no glucose added to the fetal catheters going into the chorionic plate fetal artery.

Subsequently, experiments were performed using 0.8 μCi/l of ^{14}C-L-glucose as a measure of paracellular diffusion and 8 μCi/l ^{3}H-3MG were perfused into the maternal intervillous space or the fetal artery. L-glucose is not transported by GLUT1 [8]. The non-metabolisable glucose analogue 3MG is reported to have a K_M of 1.8 mmol/l in human erythrocytes in line with the consensus values for the K_M of D-glucose [17], although a higher K_M is reported in *Xenopus* oocytes of 17.6 mmol/l for 3MG which is again comparable to the K_M D-glucose of 17 mmol/l in this system [8]. In the maternal-side tracer experiments maternal and fetal arterial D-glucose concentrations were: 0:0, 3:0, 6:0, 9:0, 9:3, 6:3, 3:3, 0:3, 0:6, 3:6, 6:6, 9:6 and 12:6 (maternal:fetal (mmol/l)) for 20 min at each step. In the fetal tracer experiments maternal and fetal arterial D-glucose concentration were: 0:3, 3:3, 6:3, 9:3, 9:6, 6:6, 3:6, 0:6, 0:9, 3:9, 6:9, 9:9, 15:9 and 15:20 (maternal:fetal (mmol/l)) for 20 min at each step. Maternal and fetal venous samples were analysed by liquid scintillation counting (Packard–Perkin Elmer, Massachusetts USA) and an enzymatic glucose assay (Alpha Laboratories, Eastleigh, UK) according to the manufacturer's instructions.

4.1. Analysis and statistics

Clearance was calculated so that transfer from maternal to fetal and fetal to maternal circulations could be compared. Clearance of ^{3}H-3MG or ^{14}C-L-glucose from one circulation to the other was calculated using the formula clearance $= [V_R].F_R/[A_D]$. Where $[V_R]$ = recipient arterial tracer activity (cpm), F_R = recipient circulation flow rate, $[A_D]$ = donor circulation arterial tracer activity (cpm).

Using simple Michaelis Menten Kinetics, predicted transport curves for GLUT1, 2, 3 and 4 were generated using literature K_M values for each transporter (Fig. 1A) [7]. This calculation assumed that donor side glucose concentrations were homogeneous and equal to the arterial concentration. Michaelis Menten Kinetics were determined using the following formula: $v_0 = V_{max}[S]/(K_M+[S])$ where v_0 is the initial rate, $[S]$ is the substrate concentration, K_M is the substrate concentration at which there is the half maximal flux and V_{max} the maximal rate of uptake. Where predictions are compared to data the V_{max} of the prediction was adjusted so that the predicted and experimental data aligned in the early part of the curve.

Transporter-mediated clearance was calculated by subtracting clearance of L-glucose (representing paracellular glucose transfer) from clearance of 3MG (which is transferred by both paracellular diffusion and transcellular transport).

Maternal venous to fetal venous and fetal venous to maternal venous ratios were calculated by dividing the venous concentration in the recipient circulation by that in the donor circulation (the circulation into which the tracer or glucose was added).

Statistical analyses were carried out using PASW SPSS19 (IBM, Chicago, IL, USA). Clearance of ^{3}H-3MG and L-glucose were analysed