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Computational modelling of amino acid exchange and facilitated transport in placental membrane vesicles

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HIGHLIGHTS

- Initial rate and time course data for serine uptake in placental membrane vesicles.
- Integrated model analysisof facilitative diffusion vs obligatory exchange.
- Dependency apparent Michaelis–Menten constants on internal concentrations.
- Uptake in placental vesicles was consistent with a facilitative transport component.
- No effects of any internal endogenous substrate in vesicles were apparent.

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ABSTRACT

Placental amino acid transport is required for fetal development and impaired transport has been associated with poor fetal growth. It is well known that placental amino acid transport is mediated by a broad array of specific membrane transporters with overlapping substrate specificity. However, it is not fully understood how these transporters function, both individually and as an integrated system. We propose that mathematical modelling could help in further elucidating the underlying mechanisms of how these transporters mediate placental amino acid transport.

The aim of this work is to model the sodium independent transport of serine, which has been assumed to follow an obligatory exchange mechanism. However, previous amino acid uptake experiments in human placental microvillous plasma membrane vesicles have persistently produced results that are seemingly incompatible with such a mechanism; i.e. transport has been observed under zero-trans conditions, in the absence of internal substrates inside the vesicles to drive exchange. This observation raises two alternative hypotheses; (i) either exchange is not fully obligatory, or (ii) exchange is indeed obligatory, but an unforeseen initial concentration of amino acid substrate is present within the vesicle which could drive exchange.

To investigate these possibilities, a mathematical model for tracer uptake was developed based on carrier mediated transport, which can represent either facilitated diffusion or obligatory exchange (also referred to as uniport and antiport mechanisms, respectively). In vitro measurements of serine uptake by placental microvillous membrane vesicles were carried out and the model applied to interpret the results based on the measured apparent Michaelis–Menten parameters K_m and V_{max}. In addition, based on model predictions, a new time series experiment was implemented to distinguish the hypothesised transporter mechanisms. Analysis of the results indicated the presence of a facilitated transport component, while based on the model no evidence for substantial levels of endogenous amino acids within the vesicle was found.

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1. Introduction

Amino acid transfer across the placenta is an important determinant of fetal growth (Jansson et al., 2006; Paolini et al., 2001; Sibley et al., 2010). Impaired fetal growth is associated with poor neonatal outcomes and in adult life with increased rates of chronic disease (Lewis et al., 2012). While currently no interventions are available for growth restricted fetuses in utero, it is known that transfer of amino acids and other nutrients across the placenta is decreased in affected pregnancies (Paolini et al., 2001) and that activity of certain amino acid transport mechanisms is impaired (Glazier et al., 1997; Jansson and Powell, 2006; Sibley et al., 1997). Hence, an improved mechanistic understanding of placental transport could potentially lead to the development of targeted treatments to either prevent or alleviate intrauterine growth restriction.

Transfer of amino acids from the maternal blood, across the placenta and into the fetal blood, is a complex process in which amino acids need to cross both the maternal facing microvillous plasma membrane (MVM) and the fetal facing basal plasma membrane (BM) of the placental syncytiotrophoblast (Cleal and Lewis, 2008; Cleal et al., 2011). Transport of amino acids is mediated by specific membrane transporter proteins. These include: (i) Accumulative transporters, which can transport amino acids against their gradient using secondary active transport driven by the sodium electrochemical gradient, thereby building up high concentrations in the syncytiotrophoblast (Philipps et al., 1978). (ii) Exchangers (antiporters), which transfer one amino acid from outside of the plasma membrane in exchange for an amino acid from inside the cytosol. Thus, exchangers play an important role in altering the composition of amino acids, but not the net amount of amino acid transferred across the placenta. (iii) Facilitated transporters, which enable facilitated diffusion of amino acids down the prevailing concentration gradient, from the placental syncytiotrophoblast into the fetal circulation, resulting in net transport.

Given this complexity, experiments using isolated plasma membrane vesicles prepared from human placental MVM or BM are commonly used to measure in vitro amino acid uptake, allowing for transporter activity to be studied under controlled conditions (Glazier and Sibley, 2006; Lewis et al., 2007). The current study will focus on the sodium-independent transport of serine, which can be primarily attributed to the transporter protein LAT2 (SLC7A8) (Lewis et al., 2007). LAT2 is believed to be an obligatory exchanger (Broer, 2008; Meier et al., 2002) although one study has reported a non-obligatory component (Segawa et al., 1999). Furthermore, in previous placental vesicle studies, sodium-independent serine uptake has been observed when amino acids were initially nominally absent inside the vesicle (zero-trans experiment) (Lewis et al., 2007). However, this is incompatible with the concept of obligatory exchange, which requires amino acid to be present on both sides of the membrane in order for exchange to occur. Therefore, this gives rise to two alternative hypotheses: (i) That sodium independent transport of serine may not be fully obligatory, or alternatively (ii), there is an initial level of endogenous amino acids present inside the vesicle, which could then enable obligatory exchange.

Mathematical modelling could potentially help to test these hypotheses (Lewis et al., 2013). Previous placental modelling studies have mainly focussed on blood flow, oxygen transfer, and solute transport by simple diffusion (Chernyavsky et al., 2010; Gill et al., 2011). Placental models including relationships for membrane transport have been applied to model transport of drugs (Staud et al., 2006) and glucose (Barta and Drugan, 2010), but few modelling studies have specifically addressed the issue of placental amino acid transport (Sengers et al., 2010). Kinetic models for carrier-mediated solute transport by membrane transporters in general have been studied extensively in the past (Friedman, 2008; Läuger, 1991; Stein and Lieb, 1986). In addition, more recent advances in computational analysis have allowed simulation of transporter function based on knowledge of the detailed molecular structure (Khalili-Araghi et al., 2009). Nonetheless, in biological experiments, the well-known Michaelis–Menten equation is most commonly applied to describe saturable transport processes (Jóźwik et al., 2004; Lewis et al., 2007; Meier et al., 2002). However, this equation does not fully represent many important transport phenomena, for instance facilitated diffusion and exchange transporters, which are intrinsically dependent on substrate concentrations on both sides of the plasma membrane. Thus, while this approach is useful to describe apparent transport properties under specific conditions (e.g. initial uptake rates), more complex mechanistic models are required to capture transporter behaviour under various physiological conditions.

The aim of this study was to use mathematical modelling to further elucidate the potential mechanisms of sodium-independent transport of serine in placental MVM vesicles. For this purpose, a standard vesicle experiment was carried out and interpreted using the model. Subsequently, model predictions based on this data were used to inform additional time-course experiments and analyse the results.

2. Methods

2.1. Transporter model

It was assumed that the kinetics of amino acid transport across the placental MVM could be described by a carrier-mediated process (Friedman, 2008; Stein and Lieb, 1986; Turner, 1983). An amino acid cannot traverse the cell membrane on its own, but needs to bind to a specific transport protein (Cleal and Lewis, 2008). Once the amino acid is bound, the transporter (carrier) can undergo a conformational change, exposing the substrate binding site to the other side of the plasma membrane to allow for transport across. Depending on the assumptions made, the carrier model can represent both amino acid transport mediated by obligatory exchangers, as well as non-obligatory (facilitative) transporters.

An extensive treatment of carrier models can be found in the reference work by Stein (Stein and Lieb, 1986). Clarification of the underlying assumptions that apply to our model is presented in Appendix A. An overview of the current model is presented in Fig. 1. Radiolabelled substrate was used experimentally to measure uptake, while unlabelled substrate can either be present inherently, or added as part of the experimental design. Therefore, radiolabelled substrate A and unlabelled substrate B were distinguished explicitly in the model. The transporter, designated as unbound carrier X, can adopt two alternative states I and II, with a binding site exposed either on the outside I or inside II of the membrane. Amino acids A and B can bind reversibly to the transporter X to form a bound substrate-carrier complex, AX or BX, which itself can also alternate between the outside and inside of the plasma membrane (Fig. 1). It was assumed that each carrier could only bind a single amino acid molecule at any one time (Fotiadis et al., 2013).

A number of simplifying assumptions were made to reduce the number of parameters to the lowest possible to represent the main features of the proposed transport mechanism. The radiolabelled amino acid A, and unlabelled amino acid B were assumed to have identical transport characteristics. The translocation rate constants for the loaded transporter complex were assumed to be equal in forward and backward directions, both given by the rate constant k. This then also implied the same binding affinity on the inside and outside of the membrane, i.e. equal dissociation constants K, based on thermodynamic arguments (Appendix A). The bound and unbound carriers do not necessarily transfer at the same rate.