System β and System A amino acid transporters in the feline endotheliochorial placenta


IT IS WELL ESTABLISHED that the amino acid supply to the human fetus across the hemomonochorial placenta is vital for adequate fetal nutrition and subsequent growth and development, in particular via the Na⁺-dependent amino acid transporters system β and system A (42). Despite this, there have been no studies of the mechanisms of amino acid transport, or indeed transport of any solute by the cat placenta, or any other species with endotheliochorial placentation.

The gross structure of the hemomonochorial human placenta is that of a branched chorionic villous tree that is bathed in maternal blood (6). It is this villous tree that provides the large surface area for maternofetal nutrient transfer. Within each mature villus, there are two cell layers that separate the fetal circulation from the maternal circulation: the fetal capillary endothelium and the trophoblast. The latter is composed of syncytiotrophoblast and cytotrophoblast cells (43). The endothelium of the fetal capillaries allows relatively unrestricted transfer of amino acids, as 15-nm spaces lie between each cell (21, 43). It is the microvillous plasma membrane (MVM; maternal facing) and basal plasma membrane (BM; fetal facing) of the syncytiotrophoblast cell layer that pose the significant barriers to placental solute transfer (21). The structure of the endotheliochorial cat placenta is quite different, and it is the lamellae of the chorioallantoic area that are the location of maternofetal exchange (32). Within each lamella, there are five tissues that separate the maternal and fetal circulations: the maternal capillary endothelium, the interstitial matrix, the trophoblast, which includes the syncytiotrophoblast and some persistent cytotrophoblast cells, the fetal stroma, and fetal capillary endothelium (28). It is the trophoblast layer that is assumed to be the rate-limiting barrier to nutrient transfer, but none of the cell layers have been investigated in terms of transport function. Therefore, as the primary barrier to solute transfer remains unexplored in the cat placenta, we chose to use an experimental model in which cellular architecture and cell-to-cell interactions are preserved for the first studies to characterize amino acid transport in this endotheliochorial placental type.

Cats lack the ability to synthesize taurine, and therefore it is an essential amino acid in this species, regardless of life stage (48). In the event of queen taurine deficiency during pregnancy, kittens receive insufficient taurine in utero and a reduced taurine content in the queen’s milk postnatally (47). This can lead to abortion, growth retardation (50), or impaired neurological function of the kittens (9, 47). Taurine is also essential to the human fetus, as its capacity to synthesize taurine via cysteine sulfinic acid decarboxylase is minimal (49). Taurine transport by system β in the hemochorial placenta has been previously examined using isolated plasma membrane vesicles (27, 31, 35, 36), perfused cotyledons (27), villous explants (44), and the trophoblast-derived choriocarcinoma JAr cell line (24, 31). System β is a Na⁺/Cl⁻/β-amino acid symport with a stoichiometry of 2:1:1 Na⁺:Cl⁻:taurine (7), which is active in MVM and to a lesser extent BM vesicles of the human placental syncytiotrophoblast (21). System β activity has been shown to be reduced in placentas from pregnancies where the fetus is growth restricted (37), indicating an important role of taurine in growth and development. System β has been cloned from human placenta (TAUT) and

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TAUT mRNA is expressed in JAr cells (39). Although taurine is essential for kittens, nothing is known of system β expression or activity in the cat placenta.

System A is the major Na⁺-dependent transporter for neutral amino acids in the syncytiotrophoblast of the human hemomonochorial placenta (21), and activity has been demonstrated in MVM vesicles (26). However, as with system β, activity is lower in BM vesicles (4, 21). System A activity in MVM vesicles is known to increase fourfold over the course of gestation. Most of this change occurs in the second trimester, correlating with the acceleration of fetal growth rate (43). System A activity has been found to be lower in placentas from small-for-gestational age (34) and intrauterine growth-restricted babies (12). When system A activity is inhibited by methyl-aminoisobutyric acid (MeAIB; a nonmetabolizable system A substrate) infusion during rat pregnancy, fetal weight is reduced (10). Three different system A isoforms have recently been cloned: ATA1 (54, 55), ATA2 (SNAT2) (17, 40, 46, 56), and ATA3 (16, 52). ATA1 and ATA2 mRNA are expressed in human placenta (17, 51, 55) and the ATA2 peptide is expressed by the MVM and BM of the human placental syncytiotrophoblast (58). Therefore, system A is an important placental amino acid transporter when considering fetal nutrition, but it has not been examined in the cat.

In this study, we have examined whether there is system β and system A amino acid transporter activity and expression in the cat placenta, using taurine (a nonmetabolizable system β substrate) and MeAIB (a nonmetabolizable system A substrate). We employed fragments of the cat placenta, which preserve the physiological relationships of the intact organ. For comparison, villous fragments of the human placenta were also investigated.

METHODS

Unless otherwise stated, all chemicals and products were obtained from Sigma (Dorset, UK) or VWR International (Dorset, UK).

Placental Fragment Uptakes

Placental collection and dissection. One cat placenta was collected from each term (64–67 days gestation), vaginal litter delivery at Waltham Centre for Pet Nutrition, Leicestershire. Full cat welfare considerations were in place. After collection, the placental girdle was transferred to control Tyrode’s buffer, pH 7.4 (135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 5.6 mM glucose) at room temperature, within 30 min. Fragments of the placental lamellar region (10–15 mg wet wt) were dissected from each cat placenta and secured with thread to metal hooks, held in Tyrode’s of differing experimental conditions. There were no systematic differences in the size of the fragments between the different experimental conditions.

At the time of collection, ethical permission was not required, as the mothers remained anonymous. Samples (1 cm³) of human placenta were dissected from the villous midline of each placenta and immersed in control Tyrode’s at room temperature within 30 min of placental delivery. Fragments of 10–15 mg wet wt, free from large blood vessels and calcium deposits, were then dissected and secured to hooks in the same way as described for the cat placenta.

Placental Fragment Uptake Protocol: Time Course

After dissection, all fragments were preequilibrated for 30 min to 37°C in 4 ml of control Tyrode’s. In the first series of experiments, a time course of uptake was determined. Uptake was measured in triplicate at 5, 10, 15, 20, and 30 min, in both the presence and absence of Na⁺, using control Tyrode’s and Na⁺-free Tyrode’s buffer, pH 7.4 [choline chloride (135 mM) replaced NaCl], respectively. A 2-min prewash in 4 ml of control or Na⁺-free Tyrode’s at 37°C was completed before uptake was initiated by immersing fragments in 4 ml of control or Na⁺-free Tyrode’s containing radiolabeled taurine or MeAIB. To terminate uptake and remove extracellular isotopes, fragments were vigorously washed, twice, in an excess (12 ml) of ice-cold control or Na⁺-free Tyrode’s for 15 s. Finally, fragments were transferred to 4 ml of dH₂O at room temperature to lyse for 16–20 h. The water lysate contained radiolabeled amino acid released from the fragments and was counted for radioactivity. Postlysing, the fragments were cut from the hooks and dissolved in 4 ml of 0.3 M NaOH at 37°C. The NaOH lysate was assayed for protein content, using Bio-Rad dye reagent concentrate (Bio-Rad Laboratories, Hertfordshire, UK) and standards of 0 to 5 μg of protein content, prepared from bovine serum albumin dissolved in 0.3 M NaOH. The NaOH lysate was then counted for radioactivity to measure the amount of radiolabeled amino acid bound to the placental fragments. The Na⁺-dependent component of amino acid uptake was calculated to represent specific carrier-mediated transport.

In the present experiments, the amount of fragment protein was used as the denominator of uptake. An ideal denominator of transport into the syncytiotrophoblast is surface area of the apical plasma membrane (AM), assuming that transport across the AM is the main contributor to uptake. However, in the absence of this information, tissue protein is considered a good proxy of membrane area (20). The size of fragments used in these experiments was kept constant to reduce differences in surface area:volume ratios between the fragments. There were no systematic differences in the size of the fragments between the different experimental conditions.

Placental Fragment Uptake Protocol: Effect of Inhibitors

Once a time course of uptake had been determined for taurine and MeAIB, transport was further characterized in an experimental series examining the effect of two inhibitors over 15 min, in the presence and absence of Na⁺. This time point was chosen, as Na⁺-dependent uptake of both taurine and MeAIB was linear at this time. Each inhibitor was added to the radiolabeled amino acid uptake medium only. The inhibitors applied were 1) an excess of a known substrate for system β or system A to act as a competitive inhibitor and 2) ouabain, an inhibitor of Na⁺-K⁺-ATPase, to dissipate the Na⁺ gradient, thereby reducing the driving force for Na⁺-dependent amino acid uptake.

Characterization of Taurine Uptake

Uptake of [³H]taurine (Amersham Pharmacia Biotech UK, Buckinghamshire, UK) was measured in fragments incubated in control or Na⁺-free Tyrode’s containing 0.4 μCi [³H]taurine (16 mM) + 10 μM “cold” taurine (human placenta only). Time course and inhibitor measurements were made as outlined above. β-Alanine served as the competitive inhibitor for system β. Fragments were incubated in triplicate in 0.4 μCi [³H]taurine containing 10 mM β-alanine or 3 mM ouabain in both control and Na⁺-free Tyrode’s. The Cl⁻ dependency of taurine uptake was also examined over 15 min. Cl⁻-free Tyrode’s buffer, pH 7.4 (MgSO₄ replaced MgCl₂ and gluconate replaced all other Cl⁻ salts), was used for the prewash and incubation solutions, as well as the ice-cold termination Tyrode’s washes, and uptake was compared with that in control Tyrode’s.

Characterization of MeAIB Uptake

A time course of [¹⁴C]MeAIB (PerkinElmer Lifesciences, Cambridge, UK) uptake was measured in placental fragments incubated in control or Na⁺-free Tyrode’s containing 0.1 μCi [¹⁴C]MeAIB (2 μM;
Cultures were maintained as described by Atkinson et al. (3) and at control for this transporter. JAr cells (European Collection of Cell protein concentration was determined using Bio-Rad dye reagent. The supernatant was recovered and centrifuged for a further 30 min at 4°C. The resulting pellet was resuspended in buffer A and protein concentration was determined using Bio-Rad dye reagent.

**Calculation of Radiolabeled Amino Acid Uptake and Statistical Analysis**

Standards of the radioactive incubation media were prepared to determine counts per minute per moles of labeled substrate using the appropriate specific activity. H₂O lystate and NaOH lystate counts were adjusted for background, divided by fragment protein (mg), divided by counts per femtomoles of taurine or MeAIB and expressed as "x" moles per milligram of placental protein per time. Results for triplicate determinations were averaged within each placenta, and data were expressed as means ± SE of n placentas. Statistical analysis using paired t-tests, a least-squares linear regression, or a repeated-measures ANOVA with Bonferroni post hoc test were performed as detailed in the figure legends.

**Western Blotting**

**Protein extractions.** Protein was extracted from cat and human placental tissue by homogenization in buffer A, pH 7.6 (10 mM HEPES, 300 mM mannitol, 1 mM 4-(2-aminoethyl)-benzenesulphonamide hydrochlorine (AEBSF) as a protease inhibitor), on ice until smooth, followed by centrifugation at 2,500 g for 5 min at 4°C. The supernatant was recovered and centrifuged for a further 30 min at 100,000 g at 4°C. The resulting pellet was resuspended in buffer A and protein concentration was determined using Bio-Rad dye reagent.

JAr cells express system β mRNA (39) and were used as a positive control for this transporter. JAr cells (European Collection of Cell Cultures) were maintained as described by Atkinson et al. (3) and at confluence were washed four times in ice-cold PBS without CaCl₂ and MgCl₂. Cells were lysed in PBS containing 1 mM AEBSF on ice for 1 h, after which the cell suspension was passed through a 21-gauge needle 10 times to yield a homogenous lysate. The cell lysate was spun at 3,210 g for 5 min and the resulting pellet was resuspended in PBS without CaCl₂ and MgCl₂ containing 1 mM AEBSF. The protein concentration was determined using Bio-Rad dye reagent. As the ATA2 peptide has previously been demonstrated in the MVM of the human placenta (58), this membrane was used as a positive control when probing for this subtype of system A. MVM protein was prepared as described previously (13).

**Sample Preparation**

For system β probing, samples were loaded with loading buffer A [20% glycerol, 300 mM Tris·HCl, pH 6.8, 0.35 M SDS, 0.25 M diethiothreitol, and 0.05% bromphenol blue (Bio-Rad Laboratories)] in a 5:1 ratio and were heat reduced for 5 min at 95°C. For system A probing, samples were mixed 1:1 with loading buffer B [22% glycerol, 139 mM Tris·HCl, pH 6.8, 154 mM SDS, 4.4 M urea (Bio-Rad Laboratories), 0.002% bromphenol blue, and 10% vol/vol 2-mercaptoethanol (Bio-Rad Laboratories)] and were heat reduced for 5 min at 95°C. All samples were loaded at 50 μg of protein and polyacrylamide gel electrophoresis was performed using 3% stacking gels and 7% resolving gels. The proteins were then electrotransferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech UK).

**Nitrocellulose Membrane Probing**

For antisystem β detection, Blotto [3% skimmed milk powder in TBS, pH 8, + 0.05% polyoxethylene sorbitan monolaurate (Tween)] was used throughout. Membranes were blocked for 1 h at room temperature in Blotto and then washed in Blotto (3 × 5 min). A rabbit antitaurine transporter affinity-purified polyclonal antibody (Chemicon International, Harrow, UK) was applied to the membranes (1:500 diluted in Blotto) at 4°C overnight. Blotto replaced the primary antibody for the negative control membrane. The following day the antibody was removed and excess was washed off using Blotto (3 × 5 min) and a secondary goat antirabbit horseradish peroxidase (HRP)-conjugated IgG antibody (DAKO, Cambridgeshire, UK) for 1 h at room temperature (1:2,000 diluted in Blotto). This was washed off in Blotto (3 × 5 min) before visualization by ECL.

For system A, the membranes were blocked and washed in Blotto as described for the system β protocol. A human anti-ATA2 antibody (kindly donated by Dr. V. Ganapathy, Department of Biochemistry and Molecular Biology, Medical College of Georgia) was then applied to the membranes (1:500 diluted in Blotto) for 1 h at room temperature. Blotto replaced the primary antibody for the negative control membrane. The antibody was washed off using TBS, pH 8, + 0.05% Tween (3 × 5 min) and a secondary goat antirabbit HRP-conjugated IgG antibody applied for 1 h at room temperature (1:2,000 diluted in Blotto). This was washed off in TBS, pH 8, + 0.05% Tween (3 × 5 min) before visualization.

**ECL Visualization**

Visualization was performed using an ECL detection system (Amersham Pharmacia Biotech UK). The molecular mass of each visualized band was interpolated from a plot of log molecular mass vs. distance migrated using kaleidoscope-prestained standards (Bio-Rad Laboratories).

**Immunohistochemistry**

Specimens of term cat (n = 3) placenta and human (n = 1) placenta were rinsed in ice-cold 0.9% NaCl before immersion fixation in ice-cold zinc fix (5). Tissue was stored at 4°C in this fixative and sent for routine processing into paraffin wax within 2 wk. Five-micrometer sections were cut using a Reichert-Jung Biocut 2030 rotary microtome and were baked onto APES-coated glass slides for 20 min at 60°C.

The localization of TAUT and ATA2 was examined using the rabbit antitaurine transporter affinity-purified polyclonal antibody and rabbit anti-ATA2 antibody that were employed for Western blot analysis. Sections were dewaxed in xylene and taken to absolute alcohol. Endogenous peroxidase activity was blocked using 400 ml methanol/2 ml hydrogen peroxide (100 vol) for 30 min. Slides were rinsed in tap water and washed in 0.05 M Tris buffer, pH 7.6 (TBS, 3 × 5 min). Nonspecific binding was blocked with serum-free protein block for 10 min and the excess was tapped off before applying the primary TAU2 antibody (1:50 dilution in TBS) or the primary ATA2 antibody (1:250 dilution in TBS) to the slides, which were left to incubate overnight at 4°C. The primary antibody was replaced with TBS on the negative control slides. The antibody was washed off using TBS (3 × 5-min washes) before applying a biotinylated secondary goat antirabbit antibody (diluted 1:200 in TBS) and leaving at room temperature for 1 h. The secondary antibody was washed away in TBS (3 × 5 min) and then 5 μg/ml of avidin peroxidase in 0.125 M TBS, pH 7.6, containing 0.347 M NaCl was applied for 1 h at room temperature. The slides were washed in TBS (3 × 5 min) and then placed in a freshly mixed bath of 280 ml of TBS and 20 ml of TBS containing 0.15 g of DAB. Immediately, 45 μl of hydrogen peroxide (100 vol) were added and the slides were left for 5 min. After this, the slides were washed under running tap water before counterstaining in 0.25% methyl green for 30 s. The slides were then rinsed again under running tap water, dehydrated, taken back to xylene, and mounted with glass coverslips. Slides were viewed under a Leitz Dialux 22 microscope.
RESULTS

System β

[^3]H]taurine uptake into cat placental fragments.[^3]H]taurine uptake was studied over a time course of 5 to 30 min in the presence and absence of Na⁺ to characterize Na⁺-dependent taurine transport in the cat placenta using the placental fragment uptake model. Figure 1A shows that[^3]H]taurine uptake by fragments of cat placenta in the presence of Na⁺ was significantly greater than in the absence of Na⁺ at all time points. When the difference between uptake in control conditions (Na⁺) and Na⁺-free conditions was calculated, a Na⁺-dependent uptake profile was derived, which was linear over 30 min (Fig. 1B). The 15-min time point was used to further investigate taurine transport characteristics in the cat placenta. Over 15 min,[^3]H]taurine uptake was significantly (P < 0.01) higher in the presence than the absence of Cl⁻ and was suppressed by 3 mM ouabain in each placenta, but the effect failed to reach statistical significance (Fig. 1C).

[^3]H]taurine uptake into human placental fragments.[^3]H]taurine uptake into the human placental fragments was investigated in the same way as in the cat placental fragments. Figure 2A shows that taurine uptake in the presence of Na⁺ was greater than in its absence at each time point. The Na⁺-dependent[^3]H]taurine uptake exhibited a linear relationship with time over 30 min (Fig. 2B). As with the cat placental fragments, the 15-min time point was selected and used to further characterize taurine transport. Figure 2C depicts the inhibitory effect of 10 mM β-alanine and 3 mM ouabain on Na⁺-dependent[^3]H]taurine uptake over 15 min. Furthermore, at 15 min,[^3]H]taurine uptake was significantly (P < 0.001) higher in the presence than the absence of Cl⁻ (data not shown), giving a Cl⁻-dependent component of taurine transport that was not significantly different in magnitude to the Na⁺-dependent component.

[^3]H]taurine bound to the placental fragments. The amount of labeled taurine that remained bound to the tissue was determined by counting the NaOH lysate for radioactivity. In all of the experiments, the amount of bound radiolabeled taurine in the presence of Na⁺ contributed less than 2% of uptake in both species under control conditions.

Expression of system β in the human and cat placenta. Term human and cat placentas were probed for system β using JAr cells as a positive control. Figure 3A shows that after a 60-min exposure, system β expression was detected in three different human and cat placental homogenate samples and in the JAr cell-positive control at ~86 kDa. In the placental tissue lanes, bands at ~32 kDa were also visible. In the negative control, also exposed for 60 min, no bands were observed (Fig. 3B).
In histological preparations of cat placenta, TAUT was localized to the fetal facing surface of each lamellae; to the BM of the syncytiotrophoblast and the fetal mesenchyme facing surface of the fetal capillary endothelium. Staining was also strongly positive throughout the decidual cells and in some endothelial cells of the maternal capillaries (Fig. 4 A). In the human placental control, TAUT was localized to the MVM of the placental syncytiotrophoblast, with some faint staining of the BM (Fig. 4 B). Negative control sections of both cat (see Fig. 9 A) and human placenta (see Fig. 9 B) completely lacked staining.

System A

\[^{14}C\]MeAIB uptake into placental fragments. \[^{14}C\]MeAIB uptake was investigated over 30 min in the presence and absence of Na\(^+\) to establish whether a Na\(^+\)-dependent system A-like transporter existed in the cat placenta. In the presence of Na\(^+\), \[^{14}C\]MeAIB uptake was greater than the absence of Na\(^+\) at 10, 15, 20, and 30 min (Fig. 5 A). Figure 5B shows that the Na\(^+\)-dependent uptake was linear over 5 to 30 min. MeAIB uptake was characterized further over 15 min. Na\(^+\)-dependent \[^{14}C\]MeAIB uptake was completely inhibited using 10 mM MeAIB (Fig. 5C). Ouabain suppressed Na\(^+\)-dependent uptake, but this failed to reach statistical significance (Fig. 5C).
suppressed uptake but this failed to reach statistical significance (Fig. 6C).

\[^{14}C\]MeAIB bound to the placental fragments. The amount of labeled MeAIB that remained bound to the tissue was
determined and in the presence of Na⁺ contributed less than 2% of uptake for both species under control conditions.

Expression of system A in the human and cat placenta. Figure 7A shows a Western blot of three different human and cat placentas probed for ATA2, along with MVM vesicles as a positive control. Bands at $\sim 57$ kDa, depicting ATA2 expression, were present in all samples after 5-min exposure; however, signal intensity in the cat placentas was much lower than that seen in either MVM or human placenta. After 5-min exposure, no bands were visible in the negative control (Fig. 7B).

In histological preparations of cat placenta, ATA2 was localized to the fetal facing surface of each lamellae only; to the BM of the syncytiotrophoblast and the fetal mesenchyme facing surface of the fetal capillary endothelium (Fig. 8A). In the human placental control, ATA2 was localized to the MVM of the placental syncytiotrophoblast, as well as within the fetal stroma (Fig. 8B). Negative control sections of both cat (Fig. 9A) and human placenta (Fig. 9B) completely lacked staining.

Fig. 6. Human placental fragment uptakes. A: $[^{14}C]$MeAIB uptake in the presence (●) and absence (○) of Na⁺ over time. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. Na⁺-free uptake; paired $t$-tests. B: Na⁺-dependent $[^{14}C]$MeAIB uptake over time, $r^2 = 0.56$, $P < 0.0001$; least-squares linear regression. C: Na⁺-dependent $[^{14}C]$MeAIB uptake in the presence and absence of 10 mM glycine and 3 mM ouabain. **$P < 0.01$ vs. Na⁺-dependent, repeated-measures ANOVA ($P < 0.01$) with Bonferroni post hoc test. Data are expressed as means ± SE (A and B), $n = 5$ placentas, means ± SE (C), $n = 7$ placentas.
DISCUSSION

In this study, we demonstrated that the cat placenta possesses two amino acid transport systems that resemble the system $\beta$ and system $A$ transporters of the human placenta, both in functional characteristics and protein expression under similar conditions.

The human and cat placental fragments that were used in this model were composed of several cell types. Measurable accumulation in this model is more representative of in vivo transport than models that measure uptake into preparations of specific cell types or membrane vesicles, as tissue architecture, cellular signaling, and driving forces all remain intact. However, results of amino acid uptake into the placental fragments reflect net accumulation into all cell types present in the tissue that express amino acid transport proteins, plus nonspecific accumulation into the extracellular space. It is assumed that specific examination of unidirectional transport was achieved in the present study by measuring uptake rate over 15 min, during which time $Na^+$-dependent uptake was linear. The regression line describing $Na^+/H^+$-dependent amino acid uptake goes through the origin when extrapolated back to the $x$, $y$-axis, consistent with measurement of uptake at initial rate. The examination of initial rate flux is important, as time-dependent amino acid uptake into placental fragments may be complicated by concurrent efflux out of the syncytiotrophoblast (57). It is assumed that uptake over the MVM into the syncytiotrophoblast compartment is predominantly measured in the human placental fragments and the predominant localization of TAUT and ATA2 to the MVM of the human placenta in the present study is consistent with this. In the cat placenta, it is not possible to distinguish over which membrane transport contributes most to total tissue accumulation. Experiments employing specific plasma membranes of the cat placenta would help to resolve this, if such a technique proves to be possible in this species. However, the immunolocalization studies carried out in the present study did address the issue of which membranes within the cat placenta possess system $\beta$ and system $A$ amino acid transporters. Both TAUT and ATA2 protein was localized to the outermost fetal facing surface of the cat placental lamellae, to the BM of the syncytiotrophoblast and to the endothelium of the fetal capillaries. The presence of TAUT and ATA2 on these membranes would act to transfer amino acids from the fetus to the placenta, but it is not possible with techniques currently available to evaluate the contribution that transport by these membranes will make to net transplacental amino acid transfer. The marked apical staining of the cat

Fig. 8. Light microscopy of term cat (A) and human (B) placenta stained for ATA2. A: ATA2 staining is present on the outermost surface of each lamellae; on the BM of the syncytiotrophoblast (long arrows) and the fetal mesenchyme facing surface of the FC endothelium (short arrows). There is also very pale staining of the D, but the MC are devoid of any staining. B: ATA2 staining is present on the MVM of the syncytiotrophoblast (long arrows) and within the fetal stroma (FS) but is absent from the FC. A and B: scale bar = 20 µm. (For key see Fig. 4A.)

Fig. 9. Negative control of term cat (A) and human (B) placenta for TAUT and ATA2. A: no staining is present in the term cat placenta. D, MC, FC, and the AM (short arrows) and BM (long arrows) of the syncytiotrophoblast are labeled. B: no staining is present in the term human placenta. FC and the MVM and BM of the syncytiotrophoblast are labeled. A and B: scale bar = 20 µm. (For key see Fig. 4A.)
placenta for TAUT eliminates the likelihood that the lack of AM ATA2 staining was due to restricted antibody access. Both decidual cells and maternal capillary endothelial cells were positive for TAUT. This finding parallels the localization of alkaline phosphatase to decidual and maternal endothelial cells (8) and further suggests a role for these cells in placental transport in this species.

In the human placenta, taurine uptake was both Na$^+$ and Cl$^-$ dependent, as well as $\beta$-alanine sensitive. These characteristics of taurine transport using the placental fragment uptake model are identical to the known characteristics of system $\beta$ that have been demonstrated by others in human placental membrane vesicles (27, 35, 36). As taurine uptake into the cat placenta demonstrated the same characteristics, the cat placenta clearly possesses a taurine transporter, with characteristics attributable to system $\beta$. There was a Na$^+$-independent component of taurine transport into both the cat and human placental fragments, yet there are no reported Na$^+$-independent uptake mechanisms for taurine (7). This, combined with the $\beta$-alanine insensitivity of the taurine uptake in the absence of Na$^+$ (data not shown), is consistent with Na$^+$-independent transport occurring by nontransporter-mediated transfer, perhaps nonspecific diffusion into the damaged areas of the fragments.

In the present study, ouabain lowered Na$^+$-dependent taurine uptake into cat placental fragments. Schmidt (41) also observed that taurine uptake into the cat retina was sensitive to ouabain inhibition. Significant (at the 5% level) inhibition of uptake was demonstrated in the human placental taurine uptakes. An explanation for the small but significant effect of uptake was demonstrated in the human placental taurine uptake in the absence of Na$^+$ (data not shown), is consistent with Na$^+$-independent transport occurring by nontransporter-mediated transfer, perhaps nonspecific diffusion into the damaged areas of the fragments.

The expression of TAUT protein in human and cat placenta supports the functional findings of this study. It confirms that system $\beta$ is present in the cat placenta and suggests that the sequence to which the TAUT antibody was raised is conserved in both the human hemomonochorial placental and the feline endotheliochorial placenta. The feline cross-reactivity of the antibody used is perhaps not surprising when considering that TAUT has 80% homology with canine, mouse, and rat taurine transporter sequences (39). Furthermore, the detection of an immunoreactive band of the same size in feline kidney as placenta (data not shown) confirms feline reactivity to this antibody and extends its application, in that this epitope is conserved both within and between species.

The Na$^+$-dependent uptake of MeAIB observed in the cat placental fragments, similar to that seen in the human, provides good functional evidence for the presence of system A in the endotheliochorial tissue. The data for the human placenta are in agreement with several previously reported observations of functional evidence for system A in this organ (11, 14, 20–22, 29, 34, 45). There was a difference in shape of the time course uptake curves for MeAIB between the cat and human placenta. MeAIB uptake in the presence of Na$^+$ into the cat placental fragments appeared to plateau toward 30 min. It is inappropriate to make quantitative comparisons of uptake (expressed per mg protein) between the two species because of the different tissue complexities. However, it is clear that the difference between MeAIB uptake in control and Na$^+$-free conditions is greater in the human than the cat placenta. Although the Na$^+$-dependent component in the cat is relatively low it is completely inhibited by 10 mM “cold” MeAIB, suggesting that this component is attributable to system A-mediated transport. In the present study, ouabain suppressed Na$^+$-dependent MeAIB uptake into cat and human placental fragments, and although this was not significant at the 5% level, it does implicate a role for the Na$^+$ gradient in driving transport by system A. The ability of ouabain to inhibit taurine uptake into human placenta to a greater extent than MeAIB might reflect a higher dependence of system $\beta$ than system A on the electrochemical gradient for Na$^+$.

As for system $\beta$, there was a Na$^+$-independent component of MeAIB uptake into the placental fragments. This cannot be attributed to system L, a Na$^+$-independent transporter of neutral amino acids functional in placenta, as it does not support MeAIB as a substrate (4). Therefore, it is probable that Na$^+$-independent MeAIB uptake is due to noncotransporter-mediated transfer into the fragments, such as diffusion into the damaged areas of the fragments. However, it is possible that the larger contribution of Na$^+$-independent uptake to total uptake in the cat compared with the human placenta might be related to the expression of a novel Na$^+$-independent carrier-mediated uptake process(es) for MeAIB in the cat. A study of glycine uptake into human placental MVM vesicles identified system A as the major route for placental glycine transport (2). In the present study, 10 mM glycine significantly inhibited MeAIB uptake into the human placental fragments and just over 20% of uptake remained glycine insensitive. The inhibition of MeAIB uptake by glycine confirms that these amino acids share a common pathway that is likely to be system A.

The ATA2 antibody proved feline cross-reactive. The expression of ATA2 protein supports the functional data showing system A (Na$^+$-dependent MeAIB uptake) activity in the
human and placental fragments and demonstrates that the ATA2 sequence to which this antibody was raised must be conserved in both the human hemomonochorial placenta and the feline endotheliochorial placenta. However, other isoforms may also contribute to uptake as both ATA1 and ATA2 genes are expressed in the human placenta (17, 51, 55).

The predicted molecular mass of ATA2 is \(~56\) kDa for rat ATA2 (51) and human ATA2 (17). The 57-kDa protein band detected in the present study is consistent with this and also with Zamudio et al. (58), who reported a 53-kDa band in the MVM and BM of human placental syncytiotrophoblast. The bands of lower molecular mass (\(~37\) kDa) detected in the human placental lanes were only apparent after longer exposure times, required to clearly detect immunoreactive bands in the cat placenta. The identity of this smaller band, which was absent from the negative control, is uncertain, but a \(~40\)-kDa band was detected by Hyde et al. (18) alongside a \(~60\)-kDa band when probing rat skeletal muscle L6 cells for rat ATA2. The smaller mass band could represent proteolytic breakdown products of ATA2, but they were not confined to placental tissue homogenates, also being apparent in MVM.

In summary, this study provides the first functional evidence of specific solute uptake mechanisms into an endotheliochorial placenta and demonstrates the usefulness of a placental fragment uptake model in such studies. It has been demonstrated that the cat placenta possesses amino acid transport systems that are functionally similar to the system \(\beta\) and system A transporters of the human placenta.

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