Human placental taurine transporter in uncomplicated and IUGR pregnancies: cellular localization, protein expression, and regulation

S. Roos, T. L. Powell, and T. Jansson
Perinatal Center, Department of Physiology and Pharmacology, Göteborg University, S-405 30 Göteborg, Sweden
Submitted 6 April 2004; accepted in final form 25 May 2004

Roos, S., T. L. Powell, and T. Jansson. Human placental taurine transporter in uncomplicated and IUGR pregnancies: cellular localization, protein expression, and regulation. Am J Physiol Regul Integr Comp Physiol 287: R886–R893, 2004. First published May 27, 2004; 10.1152/ajpregu.00232.2004.—Transplacental transfer is the fetus’ primary source of taurine, an essential amino acid during fetal life. In intrauterine growth restriction (IUGR), placental transport capacity of taurine is reduced and fetal taurine levels are decreased. We characterized the protein expression of the taurine transporter (TAUT) in human placenta using immunocytochemistry and Western blotting, tested the hypothesis that placental protein expression of TAUT is reduced in IUGR, and investigated TAUT regulation by measuring the Na⁺-dependent taurine uptake in primary villous fragments after 1 h of incubation with different effectors. TAUT was primarily localized in the syncytiotrophoblast microvillous plasma membrane (MVM). TAUT was detected as a single 70-kDa band, and MVM TAUT expression was unaltered in IUGR. The PKC activator PMA and the nitric oxide (NO) donor 3-morpholinosydnonimine decreased TAUT activity (P < 0.05, n = 7–15). However, none of the tested hormones, e.g., leptin and growth hormone, altered TAUT activity significantly. PKC activity measured in MVM from control and IUGR placentas was not different. In conclusion, syncytiotrophoblast TAUT is strongly polarized to the maternal-facing plasma membrane. MVM TAUT expression is unaltered in IUGR, suggesting that the reduced MVM taurine transport in IUGR is due to changes in transporter activity. NO release downregulates placental TAUT activity, and it has previously been shown that IUGR is associated with increased fetoplacental NO levels. NO may therefore play an important role in downregulating MVM TAUT activity in IUGR.

fetal growth; hormones; nitric oxide; placental transport; phorbol 12-myristate 13-acetate

The b-amino acid taurine (2-aminoethanesulfonic acid) is the most abundant free amino acid in a number of tissues (11) and is not incorporated into proteins. Numerous intracellular and extracellular functions have been described for taurine, including conjugation with bile acids, defense against oxygen free radicals, regulation of neuronal excitability, and involvement in cell volume regulation (11, 14, 20, 48). The main dietary sources of taurine are meat and fish, but taurine can also be synthesized de novo from cysteine in humans. However, during fetal life, taurine can be regarded as an essential amino acid, because the capacity to synthesize taurine is low or absent in the human fetus, inasmuch as it lacks the enzyme cysteine-sulfenic acid decarboxylase (11). Transplacental transfer of taurine from maternal blood to the fetal capillaries is thus the primary source of this important amino acid for the fetus. Normal fetal and neonatal development is critically dependent on the sufficient availability of taurine. It has been observed in animal experiments that taurine deficiency during pregnancy and lactation is associated with growth failure, abnormal cellular development, retinal degeneration, cardiac damage, and dysfunction of the central nervous system (19, 56, 57).

Taurine transport across the syncytiotrophoblast, the transporting epithelium of the human placenta, involves at least two fundamental steps: 1) the uptake from the maternal circulation across the microvillous plasma membrane (MVM) and 2) the transport out of the trophoblast, across the basal plasma membrane (BM) and into the umbilical circulation (3). The human taurine transporter (TAUT) has been cloned from several human tissues, including the placenta. Ramamoorthy and co-workers (50) showed that it specifically recognizes taurine and other b-amino acids and its catalytic activity is dependent on Na⁺ as well as Cl⁻ with a 2:1:1 Na⁺-Cl⁻-taurine stoichiometry. TAUT has a molecular mass of ~70 kDa, and it possesses six recognition sites for PKC-dependent phosphorylation (50). Norberg and collaborators (46) showed that the Na⁺-dependent uptake of taurine in BM is only 6% of that of MVM, demonstrating that the system is almost exclusively polarized to the MVM. Although not characterized in detail, Na⁺-independent pathways driven by the steep outwardly directed taurine gradient mediate taurine transport across BM in the fetal direction.

Intrauterine growth restriction (IUGR) can be characterized as a condition where the fetus has failed to achieve its genotypical growth potential. IUGR is associated with increased perinatal morbidity, higher incidence of neurodevelopmental impairment, and increased risk for a number of diseases in adulthood, such as cardiovascular disease and diabetes (4, 15). The altered growth pattern seen in many cases of IUGR is believed to be caused by impaired placental transport functions and blood flow (24). Norberg and colleagues (46) showed that the MVM activity of TAUT is reduced in IUGR, providing a possible mechanism for the low fetal plasma concentrations of taurine associated with IUGR (9, 12). Whether this reduction is caused by an altered number of transporters or a decrease in the affinity of the transporter for its substrate and/or a decrease in the translocation rate of the transporter-substrate complex is yet to be determined. Furthermore, it remains to be established which factors downregulate MVM taurine transport in IUGR.

Regulation of the taurine transporter has been studied primarily in cell culture models. TAUT activity has been shown to be regulated at the transcriptional or posttranscriptional level by cytokines (10, 29, 41, 53), glucose (55), nitric oxide (NO) (7, 31), p23 (18), PKC (33), and WT1 (17). PKC activation

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
seems to be one of the major factors that downregulate TAUT activity at a posttranscriptional level (6, 16, 36, 58). Kulantaivel and colleagues (33) demonstrated that the human placental choriocarcinoma cell line JAR expresses a taurine transporter with characteristics similar to those of the taurotine transporter described in normal full-term placentas and that this transporter is inhibited by activation of PKC.

The first aim of this study was to characterize the protein expression of TAUT in the human placenta. First, we studied the cellular localization of the taurine transporter using immunocytochemistry and compared relative protein densities of TAUT in isolated MVM and BM by Western blotting. Second, we tested the hypothesis that IUGR is associated with a reduced protein expression of TAUT in MVM. Third, we investigated the regulation of the syncytiotrophoblast TAUT by measuring Na\(^+\)-dependent taurine uptake in single primary villous fragments of human placenta after 1 h of incubation with different effectors. The effectors were hormones with reported alterations in fetal or maternal plasma concentrations in IUGR [growth hormone (GH), leptin, IGF-I, IGF-II, and EGF] or factors shown to regulate TAUT in other tissues [PMA, chelerythrine (CHT), TNF-\(\alpha\), IL-1\(\beta\), IL-6, 3-morpholinosydnonimine (SIN-1), and glucose]. We also assessed the effect of the immunosuppressant rapamycin, which inhibits mammalian target of rapamycin. The mammalian target of rapamycin-signaling pathway controls protein translation in response to nutrient stimuli (22). Finally, we tested the hypothesis that PKC activity is upregulated in placentas from pregnancies complicated by IUGR.

**MATERIALS AND METHODS**

**Materials.** All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), except [\(\text{\textsuperscript{3}H}\)]taurine (specific activity 31–32 Ci/mmol), which was obtained from Amersham Biosciences (Buckinghamshire, UK), and TNF-\(\alpha\) and IL-1\(\beta\), which were purchased from Calbiochem (San Diego, CA).

**Tissue collection.** Collection of placental tissue was approved by the Committee for Research Ethics at Göteborg University and was carried out with informed consent. Early-second-trimester placental tissue was obtained at terminations, and placentas were obtained from uncomplicated and IUGR pregnancies after vaginal or cesarean delivery at full term. The de

**Isolation of membrane vesicles.** The microvillous and basal membranes of the syncytiotrophoblast were isolated from human placenta according to a method described previously (21, 26). Briefly, the placenta was placed on ice immediately after delivery. Vesicle preparation started within 30 min. Centrifugations were carried out at 4°C, and all other steps were conducted on ice. The placenta was dissected, and decidua, chorionic plate, and amniotic sac were removed. Approximately 100 g of villous tissue were then cut into small pieces and rinsed in ice-cold physiological saline to remove blood. Tissue was placed in buffer D [250 mM sucrose, 10 mM HEPES-Tris, pH 7.4 at 4°C, protease inhibitors (0.7 \(\mu\)M pepstatin A, 1.6 \(\mu\)M antipain, and 80 \(\mu\)M aprotonin), and 1 mM EDTA] and homogenized. The homogenate was centrifuged at 10,000 \(g\) for 15 min, the supernatant was collected, and the pellet was resuspended in buffer D and centrifuged again at 10,000 \(g\) for 15 min. The two resulting supernatants were combined and centrifuged at 125,000 \(g\) for 30 min. The pellet crude membrane fraction was resuspended in buffer D, and 12 mM MgCl\(_2\) was added. The mixture was stirred slowly on ice for 20 min and then centrifuged at 2,500 \(g\) for 10 min. The supernatant, containing MVM vesicles, was centrifuged at 125,000 \(g\) for 30 min, and the pellets were resuspended in an appropriate volume of buffer D to give a final protein concentration of 5–10 mg/ml. Vesicle suspension was aliquoted, snap frozen in liquid nitrogen, and stored at −80°C until use.

**Measurement of protein concentration.** All protein concentrations were determined according to the method of Bradford (5) using a protein assay procedure (Bio-Rad, Hercules, CA) and BSA as the standard.

**Western blotting.** The proteins were separated by SDS-PAGE as described previously by Johansson et al. (26), with minor modifications. Vesicle suspension was thawed on ice and diluted in buffer D and sample buffer [8 M urea, 170 mM (5%) SDS, 0.04 U of bromphenol blue, 250 mM DTT, and 50 mM Tris-HCl, pH 6.8] to a final protein concentration of 1 mg/ml. The samples were heated to 95°C for 5 min and then cooled on ice. Fifteen micrograms of vesicle protein and appropriate prestained molecular weight markers were loaded on a 7% SDS-polyacrylamide gel. Electrophoresis was carried out at 200 V. The gels were then equilibrated in transfer buffer for 30 min under gentle agitation. Nitrocellulose transfer membranes, cut in the same sizes as the gels, were soaked in distilled water and then equilibrated in transfer buffer for 10 min. The gels were then transferred overnight at 30 V. To minimize unspecific binding, the membranes were blocked in 5% Blotto buffer [0.1 M PBS, 0.1% Tween 20 (vol/vol), and 5% nonfat dry milk (wt/vol)] for 1 h. After the membranes were washed in PBS-Tween, the primary rabbit antiauxine transporter polyclonal antibody (Chemicon, Temecula, CA) diluted 1:4,000 in PBS-Tween was added, and membranes were incubated overnight at 4°C. The membranes were washed in PBS-Tween before incubation in secondary horseradish peroxidase-labeled goat anti-rabbit antibody diluted 1:1,000 (Vector Laboratories) for 1 h at room temperature. After another round of washing in PBS-Tween, enhanced chemiluminescence Western blotting detection reagents (Amersham) were used to visualize the taurine transporter bands on autoradiographic film. Relative density of the bands was evaluated by densitometry with IP Lab Gel (Signal Analytics, Vienna, VA) or...
PLACENTAL TAURINE TRANSPORT

Image Gauge software (version 3.45, Fuji Film). The mean density of the control bands was an arbitrary value of 1, and the mean density of the IUGR group could then be compared with the control group.

Measurement of taurine transporter activity. Taurine transporter activity was measured according to a method developed for the system A amino acid transporter (23). Tissue from newly delivered placenta was collected and dissected into small (≈5-mm³) pieces and washed in physiological saline and placed in 1:3 DMEM-Tyrode solution, 135 mM NaCl, 5.0 mM KCl, 1.8 mM CaCl₂, 2.0 mM MgCl₂, 10 mM HEPES, and 5.6 mM D-glucose, pH 7.4) at room temperature for transport to the laboratory. Fragments were placed in fresh DMEM-Tyrode solution and further dissected into ~1-mm³ pieces. Subsequently, the fragments were tied to one end of a silk suture, and the other end was attached to a triplicate of specially designed hooks. The villous fragments were then incubated in 1:3 DMEM-Tyrode solution (pH 7.4 at 37°C), with or without the effectors under study, for 1 h. Incubations were carried out in 4 ml of buffer at 37°C. Taurine uptake experiments were carried out in Tyrode solution only, allowing easy replacement of Na⁺ by Na⁺-free incubation buffer. In Na⁺-free Tyrode solution, 135 mM choline chloride was used to replace Na⁺. To assess the substrate specificity of the transport system mediating taurine uptake, the uptake in the presence of 1 mM β-alanine (a system β substrate) was measured. Taurine transporter activity was then determined by subtracting the uptake in the control Na⁺-free buffer from the uptake in the medium containing β-alanine and Na⁺. In experiments assessing the role of Cl⁻ for the activity of the transporter, MgSO₄ replaced MgCl₂ and gluconate replaced all other Cl⁻ salts. Two sets of triplicates were studied at the same time, one incubated in Tyrode solution containing Na⁺ and the other in Na⁺-free Tyrode solution. The uptake in the Na⁺-free buffer represents nonspecific binding and Na⁺-independent uptake only. After incubation with the effectors, the fragments were washed in Tyrode solution (with or without Na⁺) for 2 min under constant agitation and then incubated for 20 min in Tyrode solution (with or without Na⁺) containing [³H]taurine (final concentration 25 nM). The fragments were then placed in ice-cold Na⁺-free Tyrode solution to stop the uptake. To lyse the cells, fragments were placed in distilled water overnight to release the [³H]taurine taken up by the cells into the water. On the next morning, the fragments were transferred to 0.3 M NaOH and left overnight. Scintillation fluid was added to the vials containing distilled water, mixed thoroughly, and counted in a scintillation counter. After denaturation in NaOH overnight, the protein concentration was measured. [³H]taurine uptake could be calculated as femtomoles of taurine per milligram of protein per 20 min. Taurine transporter activity was determined by subtracting the uptake in the Na⁺-free buffers from the uptake in the medium containing Na⁺.

Effectors. The effect of PMA, CHT, IGF-I, leptin, GH, TNF-α, IL-1β, IL-6, EGF, rapamycin, glucose, SIN-1, and IGF-II was studied. PMA, CHT, and rapamycin were prepared in DMSO and aliquoted and stored at −20°C until use. Leptin was diluted in 15 mM HCl and 7.5 mM NaOH and stored at −20°C. GH was dissolved in 10 mM Tris buffer containing 25 mM NaCl, 0.3% BSA, and 0.015% thimerosal, pH 7.4, and stored at 4°C for ≤1 mo. IGF-I was diluted in 10 mM HCl and aliquoted and stored at −20°C. Before each experiment, PBS, pH 7.4, with 0.1% BSA was added. EGF and IGF-II were diluted in 0.2 μm-filtered 10 mM acetic acid containing 0.1% BSA. Before each experiment, sterile-filtered PBS, pH 7.4, with 0.1% BSA was added to the IGF-II stock solution. SIN-1 was diluted in water and stored in −20°C until use. All stock solutions were diluted in DMEM-Tyrode buffer to give final concentrations of 617 ng/ml PMA, 1.920 ng/ml CHT, 500 ng/ml leptin, 500 ng/ml GH, 250 ng/ml IGF-I, 600 ng/ml EGF, 5.4 mg/ml rapamycin, 620 ng/ml glucose, 620 ng/ml SIN-1, and 250 ng/ml IGF-II and then used for incubation. The final concentration of DMSO was 0.02–0.08%. Controls (i.e., no effector added) and all samples were treated with an equal amount of the solvent. IL-6, TNF-α, and IL-1β were diluted in sterile 0.1 M PBS with 0.1% BSA, aliquoted, and stored at −20°C until use. The stock solutions were then diluted in DMEM-Tyrode buffer to give the following final concentrations: 30 ng/ml IL-6, 20 ng/ml TNF-α, and 20 ng/ml IL-1β.

For most effectors, the final working concentrations were chosen on the basis of concentrations previously shown to affect taurine uptake [PMA (33), glucose (55), TNF-α (29), CHT (58), SIN-1 (7), and IL-1β (53)]; IL-6 concentration was chosen to be in the same range as IL-1β. The concentrations of the hormones were chosen to be supraphysiological compared with circulating plasma levels in humans.

PKC activity. PKC activity was measured in MVM from normal full-term placentas and placentas from pregnancies complicated by IUGR with use of a PKC enzyme assay kit (Amersham). Vesicle suspension was thawed on ice and diluted with buffer D, pH 7.5 at 37°C, to a protein concentration of 130 μg/ml. Subsequently, 25 μl of vesicle sample were added to the assay buffer, and [³²P]ATP buffer was added according to the protocol provided by the manufacturer. The samples were incubated in a 37°C water bath for 10 min; addition of a stop buffer provided in the assay system ended the reaction. The samples were counted in a scintillation counter, and the results were calculated from the specific activity of 1.2 mM Mg[³²P]ATP salt.

Data presentation and statistics. The number of experiments (n) represents the number of different placentas studied. In the uptake study, three separate villous fragments were studied for each condition, and data were averaged to obtain a value representing that placenta. Similarly, in studies of PKC activity, experiments were performed in duplicate. Values are means ± SE. Differences between groups were evaluated statistically using the t-test or repeated-measures ANOVA followed by Dunnett’s test. P < 0.05 was considered significant. To determine whether the time course for taurine uptake and PKC phosphorylation was linear, a linear regression test was performed.

RESULTS

Clinical characteristics. Selected clinical data for appropriate-for-gestational age (AGA) and IUGR groups used in studies of MVM TAUT protein expression and PKC activity are given in Table 1. Gestational age was not different between the groups. Fetal weight was 38% lower (P < 0.05) in the IUGR group than in controls. Similarly, placental weight was reduced by 38% in the IUGR group (P < 0.05). Furthermore, IUGR was associated with a reduced ponderal index (P < 0.05), suggesting asymmetric fetal growth.

Table 2 shows clinical data for individual IUGR pregnancies. In six of eight cases, abnormal Doppler flow patterns were registered in the umbilical artery during days or weeks before delivery. Other indications of fetal compromise in the IUGR group included oligohydramnios (1 case) and asphyxia (3 cases). One of the women had preeclampsia, which could be the cause of the fetal distress in that case. These data suggest that the IUGR group represents fetuses subjected to true placental dysfunction. *P < 0.05 vs. AGA (t-test).

Table 1. Selected clinical data

<table>
<thead>
<tr>
<th>AGA</th>
<th>IUGR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>38.7±0.4</td>
</tr>
<tr>
<td>Fetal wt, g</td>
<td>3.532±130</td>
</tr>
<tr>
<td>Placental wt, g</td>
<td>626±30</td>
</tr>
<tr>
<td>Ponderal index, g/cm³</td>
<td>2.75±0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. AGA, appropriate-for-gestational age; IUGR, intrauterine growth restriction. *P < 0.05 vs. AGA (t-test).

Downloaded from http://ajpregu.physiology.org/ by 10.220.133.3 on April 7, 2017
Table 2. Clinical data for individual IUGR pregnancies

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Gestational Age, wk</th>
<th>Mode of Delivery</th>
<th>Fetal Wt, g</th>
<th>Placental Wt, g</th>
<th>Signs of Compromise</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>C</td>
<td>1,860</td>
<td>400</td>
<td>a, c</td>
</tr>
<tr>
<td>2</td>
<td>38.14</td>
<td>C</td>
<td>2,050</td>
<td>345</td>
<td>b, c</td>
</tr>
<tr>
<td>3</td>
<td>40.86</td>
<td>C</td>
<td>2,490</td>
<td>390</td>
<td>a, c</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>V</td>
<td>2,655</td>
<td>490</td>
<td>a</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>C</td>
<td>1,860</td>
<td>300</td>
<td>a</td>
</tr>
<tr>
<td>6</td>
<td>39.42</td>
<td>C</td>
<td>2,080</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>38.71</td>
<td>V</td>
<td>2,060</td>
<td>515</td>
<td>a, d</td>
</tr>
<tr>
<td>8</td>
<td>38.43</td>
<td>C</td>
<td>2,340</td>
<td>391</td>
<td>a</td>
</tr>
</tbody>
</table>

C, cesarean section; V, vaginal delivery. Signs of fetal compromise are as follows: abnormal Doppler flow patterns registered in umbilical artery days or weeks before delivery (a), oligohydramnios (b), asphyxia (c), and preeclampsia (d).

growth restriction, rather than genetically or constitutionally small babies.

Immunocytochemistry. Zinc-fixed tissue sections of early-second-trimester (13 wk of gestation; Fig. 1A) and full-term placentas (Fig. 1B) were incubated with a polyclonal rabbit antibody directed against TAUT to determine its cellular localization in the placenta. Expression of TAUT was detected primarily in MVM of the syncytiotrophoblast in early-second-trimester and full-term placentas (Fig. 1). No significant staining could be detected in the control sections (Fig. 1, inset).

Protein expression of TAUT. We investigated differences in protein expression between MVM and BM vesicles. Figure 2A demonstrates the presence of TAUT at ~70 kDa in MVM. BM vesicles expressed only a faint band at 70 kDa; however, a band also appeared at ~50 kDa (Fig. 2A). The specificity of these bands was confirmed by preincubation of the primary antibody with its blocking peptide (Chemicon), which abolished the 70- and 50-kDa bands.

We proceeded by performing Western blot analysis of syncytiotrophoblast MVM isolated from placentas of AGA and IUGR subjects (Fig. 2B). Densitometry analyses showed that the relative expression of TAUT was not significantly different between AGA (1.0 ± 0.1) and IUGR groups (1.1 ± 0.1).

TAUT activity. Uptake of taurine (25 nM) into villous fragments in the presence of Na\(^+\) was linear up to at least 30 min \((P < 0.05, n = 5, \text{linear regression})\); at 20 min, 87% of total uptake was Na\(^+\) dependent (Fig. 3). These data clearly demonstrate an Na\(^+\)-dependent taurine uptake in villous fragments. An incubation time of 20 min was subsequently chosen for activity measurements.

First, we determined that placental TAUT exhibits a specific requirement for Cl\(^-\) and that it is substrate specific (Fig. 4A). This was done by measuring the uptake of taurine in the absence of Cl\(^-\) and in the presence of 1 mM \(\beta\)-alanine (a system \(\beta\) substrate), respectively. Replacement of Cl\(^-\) with gluconate and 1 mM \(\beta\)-alanine reduced Na\(^+\)-dependent taurine uptake to very low levels \((P < 0.05, n = 6, \text{ANOVA; Fig. 4A})\).

Three sets of uptake experiments with different effectors were studied. In the first set of experiments, we investigated the effects of the three cytokines, IL-1\(\beta\), IL-6, and TNF-\(\alpha\). No significant changes were observed in taurine uptake after incubation with these effectors \((n = 6; \text{Fig. 4A})\).

In the second set of experiments, we examined the effect of EGF (600 ng/ml), rapamycin (20 ng/ml), glucose (30 mM), SIN-1 (3 mM), and IGF-II (250 ng/ml). SIN-1 decreased TAUT activity by 35% in placental villous fragments compared with control \((P < 0.05, n = 7, \text{ANOVA; Fig. 4B})\).

In the third series of experiments, fragments were incubated with PMA (617 mg/ml), CHT (1.920 ng/ml), leptin (500 ng/ml), GH (500 ng/ml), and IGF-I (250 ng/ml). PMA decreased TAUT activity by 21% in placental villous fragments compared with control \((P < 0.05, n = 15, \text{ANOVA; Fig. 4C})\). However, neither CHT nor any of the hormones altered TAUT activity significantly \((n = 15; \text{Fig. 4C})\).

Incubation with the effectors did not affect the Na\(^+\)-independent uptake in any of the three sets of experiments (data not shown).

PKC activity. PKC activity in MVM isolated from normal full-term placentas was linear up to at least 10 min \((P < 0.05, n = 3, \text{linear regression; data not shown})\); consequently, an incubation time of 10 min was chosen. No difference was observed in PKC activity in MVM isolated from AGA and

---

**Fig. 1.** Sections of zinc-fixed early-2nd-trimester (13 wk of gestation, A) and full-term (B) human placentas were stained with a polyclonal antibody (1:100) against the taurine transporter. Arrow, microvillous plasma membrane (MVM); arrowhead, basal plasma membrane (BM). Transporter was primarily detected in MVM of syncytiotrophoblasts in early-2nd-trimester and full-term placentas. Control section (inset) shows no significant background staining. Magnification, ×400 (A) and ×1,000 (B); scale bar, 10 μm.
IUGR placentas; 2,311 ± 367 and 1,804 ± 312 pmol·min⁻¹, mg protein⁻¹, respectively (n = 8).

DISCUSSION

Taurine is present in very high concentrations in mammalian tissues, often 100-fold higher than the extracellular concentrations (49). It is not incorporated into proteins because of the β-position of the amino group, but it still has a multitude of physiological functions (11). Animal experiments have shown that an adequate supply of taurine is critical for normal growth and development of the fetus (19, 56, 57). Because of low or absent activity of cysteine-sulfenic acid decarboxylase, the rate-limiting step in the taurine synthesis pathway, taurine can be regarded as an essential amino acid during fetal life (11). Placental transport is therefore the fetus’ key source of taurine.

A number of studies have suggested the presence of a single, high-affinity, Na⁺- and Cl⁻-dependent transport system selective for β-amino acids in the MVM of the polarized syncytiotrophoblasts (30, 40, 43). Norberg et al. (46) studied taurine transport in BM and showed that the Na⁺-dependent uptake of taurine in BM is only 6% of that of MVM, demonstrating that the system is almost exclusively polarized to MVM. The present study has, for the first time, examined the protein expression of TAUT in the placenta; the immunocytochemistry and Western blot results (the 70-kDa band) are in line with previous results showing a much higher Na⁺-dependent uptake in MVM than in BM.

Western blotting experiments using the anti-TAUT antibody (Chemicon) revealed two distinct bands (70 and 50 kDa) in BM. We proceeded by testing the specificity of these results by preincubating the primary antibody with its blocking peptide, which prevented detection of both bands. This indicates that the 50-kDa band is the result of specific binding of the antibody. The TAUT gene in mouse epididymis has recently been proposed to be subjected to alternative splicing (61), producing alternative transcripts, resulting in one or more proteins with shorter amino acid sequences than the 70-kDa TAUT. It is possible that the 70- and 50-kDa bands in BM are the result of alternative splicing of the TAUT gene in the...
syncytiotrophoblast and that the 50-kDa product is preferentially targeted to BM. When immunocytochemistry was performed, the antibody (16) showed no distinct staining in BM. This may be due to the fact that the antibody only binds to epitopes present only on the 70-kDa isoform. Because taurine is accumulated inside the syncytiotrophoblast cell by the MVM Na\(^+\)-taurine cotransporter, the steep outwardly directed taurine gradient probably drives the taurine transport across BM. However, because taurine is almost completely zwitterionic at physiological pH, nonmediated transport across BM is likely to be small. The possible function of the protein represented by the 50-kDa band may be to mediate the Na\(^+\)-independent taurine efflux from the syncytiotrophoblast. Interestingly, Jones and co-workers (28) demonstrated that in the polarized renal epithelial cell line LLC-PK\(_1\), TAUT in the apical membrane and in the basolateral membrane exhibits different Na\(^+\)-taurine stoichiometries. Furthermore, on the basis of these studies and the detection of two potential chromosomal sites for the taurine transporter, Ramamoorthy et al. (50) proposed that the polarized placental syncytiotrophoblast expresses two different Na\(^+\)-dependent taurine transporters.

Fetal plasma concentrations of taurine are known to be lower in pregnancies complicated by IUGR than in AGA pregnancies (9, 12). IUGR is associated with a distinct alteration in the MVM placental transport system for taurine. It was shown that Na\(^+\)-dependent taurine uptake was reduced by 34% in MVM isolated from IUGR full-term placentas, and it was suggested that the low plasma concentrations often found in IUGR fetuses could be caused by a reduced activity of placental taurine transporters (46). Because of the critical role of taurine supply in fetal growth and development (56), information on the cellular mechanism underlying reduced placental taurine transport in IUGR is important. One possible explanation for the reduced activity in the Na\(^+\)-dependent TAUT is a reduced protein expression, thus giving a reduced number of transporters. However, in this study, we have shown that the protein expression of TAUT is not altered in full-term placental MVM of IUGR babies, indicating that the reduced Na\(^+\)-dependent uptake in MVM from IUGR is caused by posttranslational regulation.

The cells have two ways of controlling taurine uptake across MVM: 1) altering the number of transporter proteins in the membrane by regulating protein synthesis and degradation and 2) producing conformational changes in the protein, which alter its affinity for ligands. Molecular cloning and characterization of taurine transporters have revealed several possible phosphorylation sites (25, 35, 50, 54, 60), lending support to the possibility of transporter modulation. Han and co-workers (16) demonstrated that Ser\(^{122}\) on the fourth intracellular segment is the critical site for PKC phosphorylation in Madin-Darby canine kidney cell taurine transporter and that this site is 100% conserved for TAUT in all species. The reduced activity of the transporters in IUGR placentas could be caused by phosphorylation of this site, but this remains to be established.

IUGR is associated with changes in fetal and/or maternal levels or placental expression/secretion of a number of hormones and growth factors, providing the rationale for most of the effectors tested in our regulation study. Fetal levels of IGF-I (13), leptin (34), and EGF (52) are reduced in IUGR. Maternal levels of GH (13) are also reduced. IUGR placentas show higher amounts of transcripts for IGF-II than normal placentas (1). There are contradictory results concerning maternal and fetal cytokine levels in IUGR. Some studies show elevated levels of cytokines, others show reduced levels, and still others show no change in cytokine production in association with IUGR (2, 27, 51).

We proceeded by measuring the Na\(^+\)-dependent taurine uptake in primary villous fragments after incubation with or without the effectors under study. The incubation time was set to 1 h, because the Western blotting data showed that MVM TAUT expression is unaltered in IUGR, suggesting that the reduction in MVM taurine transport in this pregnancy complication is not due to changes in transcription of the transporter but, rather, to changes in the activity. Our group previously validated that the fragments are structurally and functionally intact throughout the time period needed for the experiment, i.e., for up to 3 h after delivery (23). The concentrations of most effectors were chosen on the basis of concentrations previously shown to affect taurine uptake. Hormone concentrations were supraphysiological. However, for hormones produced by the syncytiotrophoblast and/or decidua, the in vivo concentrations close to the placental barrier are likely to be higher than in the general circulation.

Leptin, in the dose used in this study, has been shown to increase the activity of the system A transporter in primary villous fragments (23). However, we did not observe any effect of the hormones on taurine uptake. These findings suggest that the placental taurine transporter was not subjected to short-term (1 h) hormonal regulation.

The activity of TAUT was inhibited by the phorbol ester PMA, known to be an effective substitute for the physiologically generated diacylglycerols that activate PKC. This finding is in line with previous studies in different cell lines (6, 16, 36, 58), including the human placental JAR cell line (33). This study suggests that PKC inhibits TAUT at the posttranslational level. One hour, the time used for incubation of fragments in PMA, is likely to be too short to result in measurable effects on transporter synthesis. We speculated that an increased PKC activity in MVM in association to IUGR may contribute to the reduced taurine uptake across MVM in this pregnancy complication, but when we tested this hypothesis, no difference in PKC activity in MVM could be distinguished between AGA and IUGR groups. This indicates that PKC-mediated inhibition of TAUT may not contribute to the reduced activity of TAUT in MVM in association with IUGR.

NO is a signal molecule with diverse functions that include vasodilatation, inhibition of platelet aggregation and adhesion to the endothelium, and control of blood pressure (32). The enzymes responsible for the synthesis of NO from L-arginine are known as NO synthases (NOS) (47). Human placental syncytiotrophoblasts and villous endothelium express endothelial NOS (eNOS), and NO released from these cells is believed to be a key vasodilator within the placental vasculature (8, 44). Although there are a few reports suggesting that fetoplacental NO production is decreased in IUGR (42), most studies support an increased NOS activity in this pregnancy complication. For example, higher concentrations of plasma nitrates and nitrites (a reflection of NOS activity) have been found in fetal circulation in pregnancies complicated by IUGR (38). This may be due to the increased expression of eNOS in terminal villous vessels and stem villous vessel endothelium from IUGR placentas (45). The increased eNOS expression could be an
adaptive response to the increased resistance (59) and reduced blood flow (37) in IUGR. Our results show a 35% reduction of TAUT activity after 1 h of incubation with the NO donor SIN-1. Because IUGR is associated with higher levels of NO, this might represent a mechanism for the reduced taurine uptake across MVM in IUGR. There are reports showing that long-term incubation with SIN-1 increases taurine uptake (7). However, this effect was mediated through a change in protein and mRNA expression, in contrast to IUGR, where we have shown that there is no change in protein expression of TAUT. The apparent discrepant results suggest that the mechanisms by which NO affects the taurine transporter may be different in different tissues.

Recently, Khullar and colleagues (31) showed that SIN-1 inhibits several amino acid transport systems, including taurine, in MVM vesicles. This effect could also be seen on taurine uptake when fresh villous fragments were incubated with SIN-1. Interestingly, radical scavengers were able to prevent this effect in vesicles, suggesting that the altered transport may be caused by the formation of peroxynitrite, the superoxide derivate of NO. This possibility was supported by the formation of nitrotyrosine residues in MVM, an indicator of NO production (31). It is possible that formation of nitrotyrosine residues on the taurine transporter contributes to the decreased TAUT activity after SIN-1 administration in our study.

In summary, we have demonstrated that the TAUT protein is highly polarized to the MVM of the human syncytiotrophoblast, we have shown that there is no difference in MVM protein expression of the taurine transporter between normal placentas and placentas obtained from pregnancies complicated by IUGR, and we have demonstrated that PKC and SIN-1 inhibit taurine transporter activity. Because of the high NO levels in IUGR pregnancies, NO inhibition of TAUT may decrease placental taurine transport and contribute to reduced fetal taurine levels in this pregnancy complication. We also showed by Western blot that BM contains two translation products of the taurine transporter compatible with the possibility that two isoforms of TAUT are expressed in BM. However, further studies are required to verify this hypothesis and elucidate the functional implications of this finding.

ACKNOWLEDGMENTS

The authors thank R. W. Chesney and Dr. X. Han (University of Tennessee) for kindly donating the anti-TAUT antibody used for immunocytochemistry.

GRANTS

This study was supported by grants from the Wera Ekstroem Foundation, Swedish Research Council Grant 10838, the Swedish Diabetes Association, the Novo Nordisk Foundation, Firumurare-Barnhus-direktionen, the Ahlens Foundation, the Åke Wiberg Foundation, the Sven Jerring Foundation, and the Magnus Bergvall Foundation.

REFERENCES


