Hypoxanthine transport in the guinea pig and human placenta is a carrier-mediated process that does not involve nucleoside transporters

L. Felipe Barros, MD, PhD
Santiago, Chile

OBJECTIVE: The purpose of this study was to characterize the mechanisms involved in the placental clearance of hypoxanthine.
STUDY DESIGN: Uptake of isotope-labeled compounds was measured in the in situ perfused guinea pig placenta and in membrane vesicles isolated from the human syncytiotrophoblast.
RESULTS: In the guinea pig hypoxanthine uptake (from the fetal circulation) proceeded by a saturable (Michaelis constant ≈90 μmol/L), sodium-dependent mechanism that was inhibited by 19 mmol/L papaverine but not by 10 μmol/L nitrobenzylthioinosine or 10 mmol/L uridine. Uridine uptake was blocked by nitrobenzylthioinosine but not by papaverine or 4 mmol/L hypoxanthine. In human brush-border (maternal-facing) membrane vesicles hypoxanthine influx was sodium independent and best fitted to a saturable (Michaelis constant 290 ± 45 μmol/L) plus a linear component. Saturable influx was blocked by papaverine but not by nitrobenzylthioinosine. Uridine uptake was not affected by 4 mmol/L hypoxanthine. Mediated hypoxanthine uptake by human basal (fetal-facing) membrane vesicles was not detected.
CONCLUSION: At both placental blood-tissue interfaces hypoxanthine transport occurs through specific mechanisms that are different from the nucleoside transporters. (Am J Obstet Gynecol 1994;171:111-7.)

Key words: Hypoxanthine transport, nucleoside transport, nitrobenzylthioinosine, guinea pig placenta, human placenta

Hypoxanthine is the main precursor for trophoblastic nucleotide synthesis.[1] Generated from the rapid metabolic breakdown of adenosine, hypoxanthine is also of clinical interest, because it has been shown to accumulate in blood from asphyxiated babies and perfusate from ischemic organs.[2, 3] Its further degradation to uric acid by xanthine oxidase, when oxygen is available again, results in the production of the tissue-damaging superoxide anion (O_2-), a mechanism that has been proposed as a major contributor to hypoxic-ischemic injury in human neonates.[2, 3]

Hypoxanthine, a hydrophilic purine nucleobase, crosses most plasma membranes by facilitated diffusion. These carriers have been classified into two main groups according to their substrate specificity.[1] One type, present in several cell lines like Chinese hamster ovary and Ehrlich ascites tumor, also transports nucleosides such as adenosine and uridine. The other, well characterized in the human erythrocyte, mediates the translocation of nucleobases but not uridine or other nucleosides.[5] A previous study addressing hypoxanthine transport in the placenta was based on clearance measurements and concluded that the process was membrane limited.[6] Its nature has not been further explored.

We recently characterized a broad-specificity, low-affinity, nitrobenzylthioinosine-sensitive nucleoside transporter at both brush-border and basal plasma membranes of the human placental syncytiotrophoblast[7] and also demonstrated that a high density of nucleoside transporters exists at the placental capillary and umbilical vein endothelia.[8]

This study is an investigation of the mechanisms whereby the placenta transports hypoxanthine. Because a putative interaction between hypoxanthine (plasma level ≈10 μmol/L) and the nucleoside transporter could greatly disturb the tissue compartmentalization and the modulatory roles of adenosine (plasma level <0.05 μmol/L),[9] we focused on the functional dissection of nucleobase transport from nucleoside transport.

From the Departamento de Fisiología y Biofísica, Facultad de Medicina, Universidad de Chile.
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Reprint requests: L.F. Barros, MD, PhD, Department of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom.

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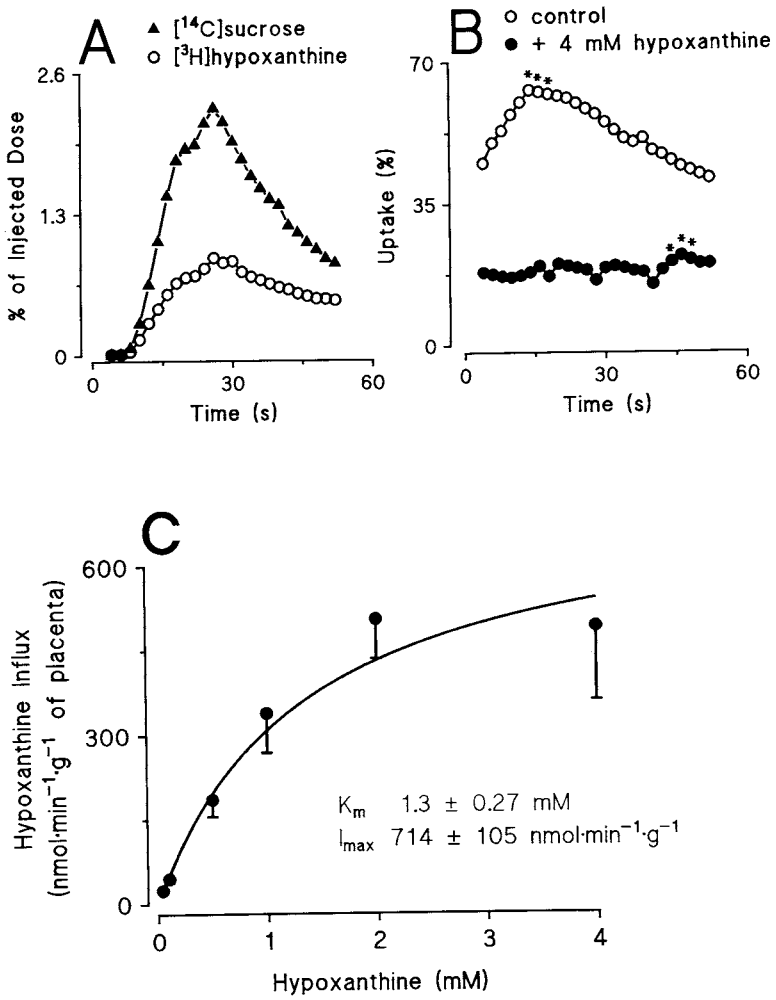

Fig. 1. Carrier-mediated hypoxanthine influx at fetal side of guinea pig placenta. **A,** Tritiated hypoxanthine and [^{14}C]sucrose activities measured in venous effluent after bolus was injected at time = 0 in umbilical artery. **B,** Upper curve shows uptake curve calculated from data in **A** according to equation 1. Lower curve was obtained in same placenta with bolus containing 4 mmol/L cold hypoxanthine. *Asterisk,* Maximal uptake is defined as average of three highest uptake values. **C,** Concentration dependence of unidirectional hypoxanthine influx. Rectangular hyperbola was fitted to mean influx measured at each concentration. Data are mean ± SEM (three placentas). Note that kinetic parameters of transport site are approximately 7% of those given here (see text). K_m, Michaelis constant; I_{max}, maximum influx.

Part of this work was published in abstract form[10] and was included in a PhD dissertation.[11]

Material and methods

Singly perfused guinea pig placenta. This preparation has been extensively described by our group.[11-13] Local animal care guidelines were followed. Briefly, white Dunkin-Hartley guinea pig dams at approximately 60 days of gestation were initially tranquilized with an intraperitoneal injection of 10 mg of diazepam and then anesthetized with sodium pentobarbital 25 to 30 mg · kg^{-1} introduced through a permanent forelimb cannula. A fetus was exteriorized through a small uterine incision, the umbilical vessels were cannulated, and the placenta was perfused at 3 ml · min^{-1} with a modified Krebs-Ringer solution. When the sodium dependence of transport was tested, sodium in the perfusate was isosmotically replaced with Tris, choline, or N-methyl-D-glucamine. Mean perfusion pressures were stable throughout the experimental period and always <30 mm Hg. The maternal circulation remained intact.

Paired tracer experiments (experimental design). Rapid unidirectional substrate uptake at the fetal interface was determined by directly comparing umbilical venous tracer concentration profiles after the arterial injection of a bolus (100 μl in about 2 seconds) containing 5 μCi of a tritiated substrate (hypoxanthine,

uridine, D-glucose, or adenine) and 0.5 μCi of carbon 14-labeled sucrose (extracellular marker). The venous effluent was sequentially sampled: 15 to 25 aliquots (100 μl) over a 60 to 80 second period. The time course of tritiated substrate uptake was determined by quantifying the uptake in each effluent sample by means of equation 1[11-13]:

$$\text{Uptake} = 1 - (\text{Tritiated substrate}/[^{14}\text{C}]\text{Reference}) \quad (1)$$

A maximal uptake value (U_{max}) was calculated by averaging the three highest values (Fig. 1). When the effect of inhibitors was measured, they were directly dissolved in the bolus unless otherwise stated. Unidirectional influx, v, was estimated from the fractional maximal uptake, the perfusion rate (F) ($ml \cdot min^{-1} \cdot gm^{-1}$ placental wet weight) and the concentration (millimoles per liter) of the unlabeled substrate in the perfusate (C):

$$v = -F \cdot \ln (1 - U_{max}) \cdot C \quad (2)$$

Preparation and characterization of human placental membrane vesicles. Syncytiotrophoblast brush-border[14] and basal membrane vesicles[15] from human term placenta obtained within 30 minutes of delivery were isolated by established techniques. Institutional approval for the use of this tissue was given. Purity of the preparations was assessed by measuring alkaline phosphatase activity as a brush-border marker and binding of tritiated dihydroalprenolol as a basal marker.[7] Compared with the initial homogenate, enrichment (fold, mean ± SEM [number of placentas]) of alkaline phosphatase activity were 17 ± 1.3 (19) for brush-border and 0.76 ± 0.16 (6) for basal membranes. Basal membranes were 22 ± 3 (4)-fold enriched in tritiated dihydroalprenolol binding sites, whereas brush-border membranes showed an enrichment factor of 1.4 ± 0.16 (4). Contamination by erythrocyte membranes in either preparation was <0.5% as determined by using the activity of acethylcholinesterase as a red blood cell marker.[16] Vesicles were resuspended in 300 mmol/L mannitol, 5 mmol/L HEPES–Tris, pH 7.4, revesiculated by passing them 20 times through a 25-gauge syringe needle, and used immediately for uptake measurements. In addition, aliquots of the membrane suspension were frozen and stored at −70° C until use. Intravesicular volumes measured with four nonmetabolized radioisotopes were 1.24 ± 0.08 (32) and 0.26 ± 0.05 (18) $\mu l \cdot mg^{-1}$ of protein for brush-border and basal membrane vesicles, respectively.

Hypoxanthine uptake and metabolism and tritiated nitrobenzylthioinosine binding in human placental vesicles. The uptake of tritiated hypoxanthine and tritiated uridine (25 $\mu Ci \cdot ml^{-1}$) at 22° C was measured by a rapid filtration technique as described,[7] with the two following modifications: (1) to maximize the signal-to-noise ratio extravesicular tritium trapped in the nitrocellulose filter was discarded by using [^{14}C]sucrose as nonpermeable marker[5] and (2) the ice-cold solution used to stop tritiated hypoxanthine uptake contained no inhibitors. Initial uptake rates were determined by fitting a monoexponential curve to the data by means of a nonlinear regression program (Enzfitter, Elsevier-Biosoft, Cambridge, U.K.) as described.[7] Metabolism of tritiated hypoxanthine by brush-border and basal membranes was negligible (<5% in 30 minutes) as assessed by thin-layer chromatography. Equilibrium binding of tritiated nitrobenzylthioinosine was determined by rapid filtration.[7]

Chemicals. Tritiated adenine (29 Ci/mmol), [G-^{3}H]hypoxanthine (8 to 12 Ci/mmol), [5,6-^{3}H]uridine (25 Ci/mmol), and tritiated glucose (20 to 30 Ci/mmol) were obtained from NEN-DuPont (Stevenage, U.K.); 1-[propyl-2,3-^{3}H]dihydroalprenolol (35 Ci/mmol) and [G-^{3}H]nitrobenzylthioinosine (23 Ci/mmol) were purchased from Amersham (Amersham, U.K.) and Moraveck Biochemicals (Brea, Calif.), respectively. Dilazep was provided by Hoffmann-La Roche (Basel, Switzerland). All other chemicals were reagent-grade quality.

Statistical analysis. Averaged results are given as mean ± SEM (n), where n is number of placentas, unless otherwise specified.

Results

Carrier-mediated hypoxanthine transport in the perfused guinea pig placenta. Fig. 1 shows representative paired-tracer data obtained with tritiated hypoxanthine and [^{14}C] sucrose. The marked difference in the venous recovery of both isotopes (Fig. 1, *A*) plus the saturability of the pathway by cold substrate (Fig. 1, *B*) suggest that the nucleobase is entering an intracellular compartment by means of a specific transporter.

Experiments were performed to determine the saturation parameters of the transport pathway. However, because flow and perfusion pressure turned out to be unstable with concentrations >100 μmol/L in the perfusate, it was necessary to adopt a less direct protocol by manipulating the concentration of cold hypoxanthine in the bolus. The peak tracer concentration in the microcirculation at the maximum uptake is ≈7% of that in the bolus.[12, 13] Michaelis-Menten parameters of hypoxanthine influx obtained with this approach are shown in Fig. 1, *C*. They suggest a Michaelis constant ≈90 μmol/L and maximum influx ≈50 $nmol \cdot min^{-1} \cdot gm^{-1}$ for the hypoxanthine transporter.

Because sodium-dependent, accumulative adenosine transporters are present in the small intestine, kidney, liver, and other organs[17] and there is evidence for accumulative hypoxanthine uptake in the choroid plexus[4] and small intestine,[18] hypoxanthine uptake was measured in the presence of different extracellular

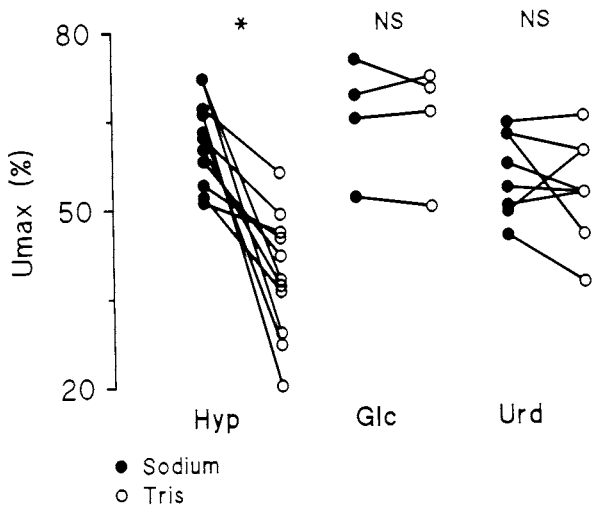

Fig. 2. Effect of extracellular sodium depletion on uptake of hypoxanthine by fetally perfused guinea pig placenta. Uptake of tritiated hypoxanthine *(Hyp)*, 3-*O*-methyl-D-[^{3}H]glucose *(Glc)*, and tritiated uridine *(Urd)* was measured in presence of either 140 mmol/L Tris or 140 mmol/L sodium in perfusate. Maximum radioactivity present in venous effluent before each injection was not higher than 0.1% of that measured in samples corresponding to maximum uptake (U_{max}). Neither perfusion flow nor pressure was affected by change of perfusate. Inhibition in absence of sodium was completely reversible. Summary of experiments performed in 11 placentas. *Asterisk*, $p < 0.05$ in Student paired t test. *NS*, Not significant.

cations. Fig. 2 shows that hypoxanthine uptake was significantly higher in the presence of sodium (61% ± 2% [12]) than in the presence of Tris (38% ± 3 [10]). The lower rate measured in Tris was not because of nonspecific placental damage, because the loss of extracellular marker was similar in sodium and Tris (8 ± 4 [12] and 10 ± 5 [11], respectively). Moreover, uptake was also lower in the presence of equimolar choline (42%, 37%) or N-methyl-D-glucamine (35%, 38%). The inhibition measured in the presence of cations other than sodium (approximately 40%) is not as strong as that obtained with cold hypoxanthine (61%, Table I). Such a discrepancy might be because of a single carrier transporting with lower efficiency in the absence of sodium (slippage) or to the presence of a second transporter that is sodium independent. Our data do not exclude either possibility. Uptake of 3-O-methyl-glucose through the sodium-independent erythroid glucose transporter GLUT-1[19, 20] was, as expected, similar in both ionic conditions. The uptake of uridine was sodium independent, which is consistent with the high density of nitrobenzylthioinosine-sensitive nucleoside transporters that are located at the fetal microcirculation.[8, 13]

Functional dissection of hypoxanthine and nucleoside transport in the guinea pig placenta. The simplest explanation for the different sodium sensitivity of hypoxanthine and uridine uptake is that they are transported by different mechanisms. Such a hypothesis was tested by measuring uptake in the presence of nucleobases, nucleosides, and nontransported compounds known to inhibit either nucleobase or nucleoside transport. The results, shown in Table I, indicate that hypoxanthine is transported by a papaverine-sensitive carrier that also recognizes the bases adenine and guanine. The lack of inhibition of this system by uridine (a substrate of all known nucleoside transporters[17]), dipyridamole, and nitrobenzylthioinosine strongly suggests that no hypoxanthine is transported by nucleoside carriers. However, the lack of inhibition of tritiated uridine uptake by hypoxanthine indicates that the nucleoside carrier present at the fetal-placental microcirculation is not affected by the nucleobase. This knowledge is instrumental to model the tissue compartmentalization of adenosine, because the plasma concentration of hypoxanthine (10 to 40 μmol/L) is 100- to 1000-fold that of the nucleoside.[9]

The inhibitory effect of high concentrations of adenosine and inosine show that, in contrast to uridine, these nucleosides do interact with the hypoxanthine carrier. This apparent discrepancy could be because hypoxanthine, adenosine, and inosine have a purine ring, whereas uridine possesses a much smaller pyrimidine ring. The interaction between adenosine and the nucleobase carrier is not followed by efficient translocation, because hypoxanthine does not affect adenosine uptake.[13] Hypoxanthine metabolically derived from adenosine or inosine should not have impeded the uptake of labeled hypoxanthine, because the fetal-placental degradation of these compounds has been shown to be intracellular.[13]

Carrier-mediated hypoxanthine transport in membrane vesicles isolated from the human placenta. Considering the microscopic structure of the hemomonochorial placenta (e.g., guinea pig and human), two cell types are most likely to be responsible for the high rate of hypoxanthine uptake found in the perfused organ: the fetal capillary endothelium and the syncytiotrophoblast. No methods have yet been reported to isolate the endothelium; thus we focused on the trophoblast plasma membranes at both maternal (brush-border) and fetal (basal) sides. These membrane preparations are well characterized in the human and have been extensively used to study the transport of a number of nutrients, including amino acids and nucleosides.[21]

Fig. 3, *A*, shows the time course of tritiated hypoxanthine uptake by basal membrane vesicles. The initial uptake was low (0.016 ± 0.005 [3] pmol · mg^{-1} · sec^{-1}) and was not inhibited by either cold hypoxanthine (2% ± 3% [3]) or papaverine (−1% ± 2% [3]) or by including 100 mmol/L choline instead of sodium in the

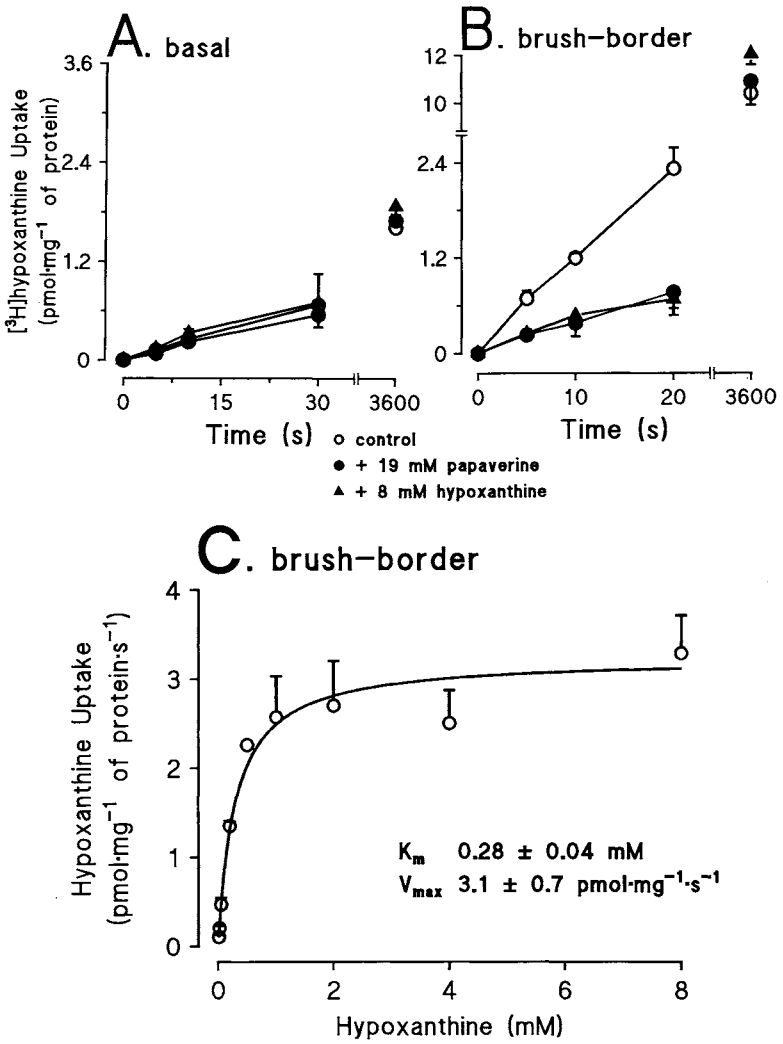

Fig. 3. Hypoxanthine uptake by brush-border and basal vesicles isolated from human placental trophoblast. Basal **(A)** or brush-border **(B)** membrane vesicles (approximately 100 μg of protein) were incubated with 6.6 μmol/L tritiated hypoxanthine and tracer amounts of [^{14}C]sucrose (extravesicular marker) in presence of inwardly directed gradient of 100 mmol/L sodium chloride. Uptake was also measured in presence of 8 mmol/L hypoxanthine or 19 mmol/L papaverine. Values are mean ± SEM of triplicate estimates in single placenta. **C,** Concentration dependence of papaverine-sensitive tritiated hypoxanthine uptake by brush-border vesicles. Data are mean ± SEM (three placentas). K_m, Michaelis constant; V_{max}, maximum velocity.

Table I. Effect of nucleobase and nucleoside-related compounds on tritiated hypoxanthine and tritiated uridine uptake by in situ perfused guinea pig placenta

Unlabeled substrate in bolus	*Inhibition of uptake (%)*			
	Tritiated hypoxanthine	*n*	*Tritiated uridine*	*n*
Hypoxanthine (4 mmol/L)	61 ± 7	11	1 ± 3	5
Adenine (4 mmol/L)	45 ± 11	4	NM	–
Guanine (120 μmol/L)	52 ± 6	2	NM	–
Papaverine (19 mmol/L)	46 ± 12	4	5 ± 4	3
Uridine (10 mmol/L)	−2 ± 4	4	70 ± 5	7
Adenosine (10 mmol/L)	38 ± 10	6	68 ± 7	4
Inosine (10 mmol/L)	40 ± 7	3	68	1
Dipyridamole (100 μmol/L)	1 ± 3	5	56, 64	2
Nitrobenzylthioinosine (10 μmol/L)	0 ± 2	3	60 ± 8	8

Unlabeled compounds were introduced as bolus together with tracer (5 μCi = 0.5 nmol in bolus) with exception of dipyridamole and nitrobenzylthioinosine, which were included in perfusate. Results are mean ± SEM. *NM*, Not measured.

Table II. Effect of inhibitors and sodium replacement on tritiated hypoxanthine and tritiated uridine uptake by placental brush-border membrane vesicles

Unlabeled substrate or condition	*Inhibition of uptake (%)*			
	Tritiated hypoxanthine (6.6 μmol/L)	*n*	*Tritiated uridine (6.6 μmol/L)*	*n*
Hypoxanthine (4 mmol/L)	60 ± 3	14	−10,0	2
Adenine (4 mmol/L)	0 ± 5	3	NM	–
Guanine (120 μmol/L)	5, 2	2	NM	–
Papaverine (19 mmol/L)	66 ± 4.8	10	5, 3	2
Uridine (10 mmol/L)	−2 ± 4	4	80 ± 5	3
Adenosine	Ki = 2.6 ± 1.0 mmol/L	3	Ki = 100 μmol/L[7]	–
Nitrobenzylthioinosine (10 μmol/L)	1.8 ± 4.5	3	80 ± 12	3
Hexanol (15 mmol/L)	−100 ± 10	3	35 ± 5	3
Hexanol (15 mmol/L) plus papaverine (19 mmol/L)	54 ± 8	3	NM	–
Hexanol (15 mmol/L) plus nitrobenzylthioinosine (10 μmol/L)	NM	–	76 ± 6	3
Sodium replaced with Tris or choline	−8 ± 3	4	NM	–

Results are mean ± SEM. Unlabeled compounds were present in isotope medium with exception of papaverine, dipyridamole, and nitrobenzylthioinosine, which were added to vesicles 30 minutes before assay. *NM*, Not measured; *Ki*, affinity constant.

incubation medium (0%, 3%). The lack of a specific signal frustrated any further characterization of hypoxanthine transport in this preparation. The quality of the basal vesicles was assessed by measuring the uptake of 50 μmol/L tritiated alanine, which was shown to be sodium dependent and was 95% inhibited by 50 mmol/L cold alanine (not shown).

When a similar protocol was applied to brush-border vesicles, a tenfold higher initial rate of uptake was measured (0.16 ± 0.04 [14] pmol · mg^{-1} · sec^{-1}). This process was sodium independent but could be inhibited by cold hypoxanthine or papaverine, which indicates the presence of carrier-mediated transport (Fig. 3, *B*, and Table II). The concentration dependence of tritiated hypoxanthine uptake by brush-border vesicles is shown in Fig. 3, *C*.

Functional dissection of hypoxanthine and uridine transport in the human placenta. The different inhibition patterns of hypoxanthine and uridine uptake suggest that in the human placenta hypoxanthine and nucleosides also follow a different route (Table II). Adenosine shows a 10 times lower affinity for the hypoxanthine carrier compared with its own transporter. Consistently 8 mmol/L hypoxanthine displaced < 10% of tritiated nitrobenzylthioinosine bound to the nucleoside transporter, whereas adenosine completely inhibited the binding, showing an affinity of 230 μmol/L (not shown). These results are also in agreement with the lack of inhibition of adenosine uptake by hypoxanthine in the guinea pig.[13]

It has been shown that some alcohols in the millimoles per liter range are good inhibitors of nucleoside transport in the erythrocyte, although they are much less effective at inhibiting glucose transport.[22] We studied the effect of hexanol, an anesthetic alcohol, on the uptake of hypoxanthine by placental membranes. As in the erythrocyte, in brush-border membranes hexanol was an effective inhibitor of uridine uptake (Table II); in contrast, a twofold increase of hypoxanthine uptake was measured in its presence. This effect was not because of an increase in the rate of simple diffusion, because nonmediated uptake assessed in the presence of papaverine was only slightly increased (Table II). This finding not only gives extra weight to the hypothesis of separate nucleoside and nucleobase carriers, it could also have mechanistic implications.

Comment

This study demonstrates for the first time the presence of carrier-mediated transport of hypoxanthine in the placenta. At both maternal and fetal blood-placental interfaces hypoxanthine uptake is mediated by low-affinity, papaverine-sensitive systems that do not transport nucleosides. On the other hand, nucleoside transport is not affected by hypoxanthine. From the physiologic point of view this independence ensures that the putative modulatory roles played by adenosine[23] are not affected by fluctuations in the concentration of the much more abundant hypoxanthine.

On the basis of their selectivity pattern the nucleobase carriers in the placenta can be included in group II according to the classification of Plagemann et al.[4] Nevertheless, some of their properties are at variance with those reported in other organs. For example, the sodium-independent hypoxanthine transporter located at the brush-border clearly differs from that of the human erythrocyte,[5] because it does not recognize adenine or guanine, purine nucleobases that are taken up by the erythrocyte carrier with high affinity.[5] LLC-PK_1, a kidney-derived epithelial cell line, also has a sodium-independent component for hypoxanthine that can be blocked by adenine.[24] The hypoxanthine carrier

present at the fetal side of the placenta is also unusual. Although the uptake is mostly sodium dependent, it can be inhibited by adenine and guanine. This is in marked contrast with the sodium-dependent component reported in LLC-PK_1 cells. Knowledge about the protein structure of these transporters is needed to understand the basis of their functional heterogeneity.

Besides the sodium-dependent carrier identified in LLC-PK_1 cells, sodium-dependent pyrimidine nucleobase uptake has been demonstrated in rat small intestine.[18] It appears then that in both kidney and intestine these active carriers are responsible for the vectorial transport recovery of nucleobases from the luminal side of the epithelia.

Our results suggest that in the placenta, also a polarized epithelial organ, the arrangement is opposite. The sodium-dependent transporter is at the basal side (fetal), whereas at the brush-border side uptake is a facilitated diffusion process. The exact cellular location of the active carrier is uncertain. Considering the much smaller size of basal vesicles compared with brush-border vesicles[14, 15] (Fig. 3, *A*), it is possible that a transport system is effectively present in the basal membrane but could not be detected because of a small signal-to-noise ratio. Experiments to isolate the placental microvascular endothelium are currently being undertaken to investigate this important point.

The results obtained in the guinea pig strongly suggest that, in vivo, the carrier at the fetal side clears up hypoxanthine coming from the fetus. In hypoxia this mechanism would limit accumulation of the nucleobase in fetal tissues. The carrier at the maternal side most probably transports hypoxanthine destined for use as a purine precursor in the placenta.[1] Because hypoxanthine carriers also transport 6-mercaptopurine[25] and 6-thioguanine,[4] drugs of widespread use in anticancer chemotherapy, these results are also of clinical interest.

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REFERENCES

1. Vettenranta K. Pathways of purine nucleotide synthesis in the human trophoblast early and late in gestation. J Dev Physiol 1988;10:547-54.
2. Ruth V, Autti-Ramo I, Granstrom M, Korkman M, Raivio KO. Prediction of perinatal brain damage by cord plasma vasopressin, erythropoietin, and hypoxanthine values. J Pediatr 1988;113:880-5.
3. Pietz J, Guttemberg N, Gluck L. Hypoxanthine: a marker for asphyxia. Obstet Gynecol 1988;72:762-6.
4. Plagemann PGW, Wohlhueter RM, Woffendin C. Nucleoside and nucleobase transport in animal cells. Biochim Biophys Acta 1988;947:405-43.
5. Domin BA, Mahoney WB, Zimmerman TP. Purine nucleobase transport in human erythrocytes. J Biol Chem 1988; 263:9276-84.
6. van Kreel BK, van Dijk JP, Pijnenburg AMCM. Placental transfer and metabolism of purines and nucleosides in the pregnant guinea pig. Placenta 1982;3:127-39.
7. Barros LF, Bustamante JC, Yudilevich DL, Jarvis SM. Adenosine transport and nitrobenzylthioinosine binding in human placental membrane vesicles from brush-border and basal sides of the trophoblast. J Membrane Biol 1991;119:151-61.
8. Barros LF, Beaumont N, Jarvis SM, et al. Immunological detection of nucleoside transporters in human placental trophoblast brush-border plasma membrane and placental capillary endothelial cell. J Physiol 1992;452:348P.
9. Maguire MH, Szabo I, Slegel P, King CR. Determination of concentration of adenosine and other purines in human term placenta by reversed-phase high-performance liquid chromatography with photodiodo-array detection: evidence for pathways of purine metabolism in the placenta. J Chromatogr 1991;575:243-53.
10. Barros LF, Yudilevich DL. Hypoxanthine transport in guinea-pig and human placenta [Abstract]. J Physiol 1991; 438:279P.
11. Barros LF. Transporte de adenosina, hipoxantina y glucosa en la placenta [Dissertation]. Ph D Santiago: University of Chile, 1993.
12. Yudilevich DL, Eaton BM, Short AH, Leichtweiss WP. Glucose carriers at maternal and fetal sides of the trophoblast in guinea-pig placenta. Am J Physiol 1979;237:C205-12.
13. Wheeler CPD, Yudilevich DL. Transport and metabolism of adenosine in the perfused guinea-pig placenta. J Physiol 1988;405:511-26.
14. Glazier JD, Jones CJP, Sibley CP. Purification and Na^+ uptake by human placental microvillous membrane vesicles prepared by three different methods. Biochim Biophys Acta 1988;945:127-34.
15. Kelley LK, Smith CH, King BF. Isolation and partial characterization of the basal cell membrane of human placental trophoblast. Biochim Biophys Acta 1983;743: 91-8.
16. Vanderpuye OA, Kelley LK, Morrison MM, Smith CH. The apical and basal plasma membranes of the placental syncytiotrophoblast contain different erythrocyte membrane protein isoforms. Evidence for placental band 3 and spectrin. Biochim Biophys Acta 1988;943:277-87.
17. Ruiz-Montasell B, Casado FJ, Felipe A, Pastor-Anglada M. Uridine transport in basolateral plasma membrane vesicles from rat liver. J Membrane Biol 1992;128:227-33.
18. Bronk JR, Hastewell JG. The transport of pyrimidines into tissue rings cut from rat small intestine. J Physiol 1987: 382:475-88.
19. Johnson LW, Smith CH. Glucose transport across the basal plasma membrane of human placental syncytiotrophoblast. Biochim Biophys Acta 1985;815:44-50.
20. Barros LF, Baldwin SA, Jarvis SM, et al. In human placenta the erythroid glucose transporter GLUT1 is abundant in the brush-border and basal membranes of the trophoblast but the insulin-dependent isoform GLUT4 is absent: neither is detectable in endothelial cells. J Physiol 1992;446:345P.
21. Yudilevich DL, Barros LF. Transport of nucleosides and aminoacids in the placenta. Biochem Soc Trans 1990;18: 1146-50.
22. Nagy LE, Diamond I, Casso DJ, Franklin C, Gordon AS. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. J Biol chem 1990;265:1946-51.
23. Schocken DD, Schneider MN. Use of multiple radioligands to characterize adenosine receptors in human placenta. Placenta 1986;7:339-48.
24. Griffith DA, Jarvis SM. Sodium-dependent and -independent nucleobase transport in cultured LLC-PK1 epithelia. Biochem Soc Trans 1992;20:242S.
25. Hashimoto H, Kubota M, Shimizu T, et al. Biochemical basis of the prevention of 6-thiopurine toxicity by the nucleobases hypoxanthine and adenine. Leukemia Res 1990;14:1061-6.