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UPTAKE OF CHOLINE INTO SYNCYTIAL MICROVILLUS MEMBRANE VESICLES OF HUMAN TERM PLACENTA

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Abstract—The uptake of the quaternary ammonium compound choline was studied in syncytial microvillus membrane vesicles of human term placenta. Uptake was stimulated by an inside negative membrane potential and by loading the vesicles with unlabeled choline. Imposition of an inwardly directed Na^+ or outwardly directed H^+ gradient did not stimulate choline uptake. Several organic cations were able to inhibit choline transport in the following order: hemicholinium-3 ≥ choline ≥ mepiperphenidol > cimetidine ≥ famotidine. The kinetics of uptake involved a saturable process for choline with high affinity (K_m = 550 μM). Our results confirm the presence of a carrier mediated transport system in human placental syncytial microvillus membranes. The system appears to be electrogenic, and able to transport choline efficiently from the maternal circulation into the placenta.

Key words: choline transport, human placenta, syncytial microvillus membrane vesicles

The cationic quaternary ammonium compound choline is an essential substrate for the synthesis of phospholipids and acetylcholine. Since the placenta and fetus do not synthesize choline, it must be transported from the maternal plasma by the trophoblast [1]. The human placental concentration of choline is approximately 1 mM, whereas maternal plasma contains about 20 μM choline. Accumulated choline against this concentration gradient is rapidly converted into acetylcholine [2]. Although acetylcholine is present in large concentrations in the human placenta its main function, in an organ which lacks innervation, remains unclear. It has been proposed that the placental cholinergic system plays a role in the regulation of amino acid transport [3].

The mechanisms of choline transport have been characterized in various tissues. In neural tissue a high affinity sodium co-transport system has been described [4]. In erythrocytes facilitated diffusion is involved, which is not sodium dependent [5]. In rat intestinal brush-border membranes a choline transporter independent of sodium, H^+ or membrane potential was found with a K_m of 159 μM [6]. Two saturable transport systems were found in rabbit renal brush-border membranes (K_m = 97 μM and $K_m \approx$ 10 mM). Uptake was stimulated by an inside negative membrane potential and by a *trans* concentration gradient of choline [7].

The mechanism of choline transport across the placenta has still not been fully elucidated. In placenta fragments choline was concentrated against a concentration gradient. Non-saturable passive diffusion in combination with carrier-mediated transport was found, with K_m = 350 μM and V_{max} = 75 nmol/mL intracellular water/min [1]. Saturable sodium independent choline uptake into the trophoblast was demonstrated in the dually perfused guinea pig placenta [8] and human placenta [9]. Non-saturable components, reflecting passive diffusion, were also observed. Driving forces and specificity, however, of choline transport in the human placenta have still not been clarified.

The present study was designed to provide insight into the mechanisms of choline transport across the human placenta, using isolated SMMV‡ of human term placenta.

MATERIALS AND METHODS

Chemicals. [^{3}H]Choline was obtained from Amersham (Aylesbury, U.K.), cimetidine from Smith, Kline & French (Welwyn Garden City, U.K.) and mepi from Merck, Sharp & Dohme (Rahway, NJ, U.S.A.). All other chemicals were purchased from either the Sigma Chemical Co. (St Louis, MO, U.S.A.) or Merck (Darmstadt, Germany) and were of analytical grade. GF/F filters were obtained from Whatman Int. Ltd (Maidstone, U.K.).

Preparation of SMMV. SMMV were prepared from fresh human term placentae, essentially according to a procedure described by Glazier *et al.* [10]. Tissue was minced in a Warring blender. The mince was stirred for 30 min to loosen the microvilli. After $MgCl_2$ aggregation and differential centrifugation SMMV were harvested and suspended in an appropriate intravesicular buffer to a final protein concentration of 10–15 mg/mL. Vesicles

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‡ Abbreviations: HC-3, hemicholinium-3; mepi, mepiperphenidol; Mes, 2(*N*-morpholino)ethanesulfonic acid; NMN, *n*-methylnicotinamide; pH_i, pH inside the vesicle; pH_o, pH outside the vesicle; SMMV, syncytial microvillus membrane vesicles; TEA, tetraethylammonium; val, valinomycin.

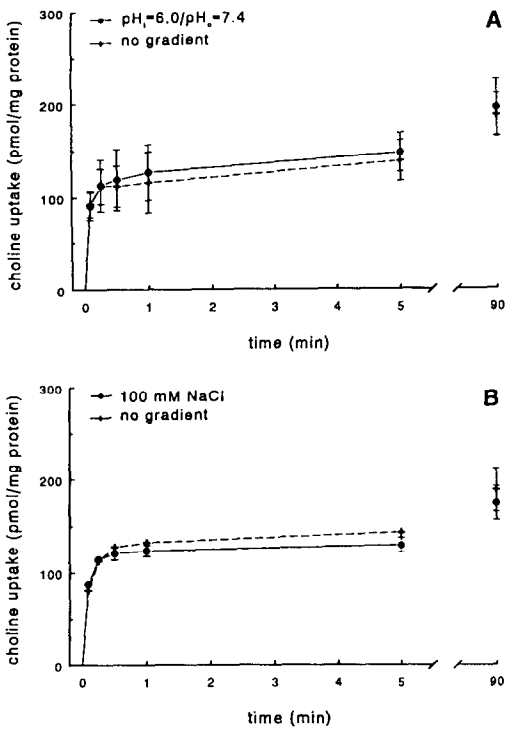

Fig. 1. Effect of an outwardly directed proton gradient (A) and inwardly directed sodium gradient (B) on the uptake of 25 μM choline into SMMV. (A) Vesicles were suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes–Tris, pH = 7.4 or 10 mM Mes–Tris, pH = 6.0. Extravesicular medium consisted of 100 mM mannitol, 100 mM KCl and 10 mM Hepes–Tris, pH = 7.4. (B) Vesicles were suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes–Tris, pH = 7.4. Extravesicular media consisted of 100 mM mannitol, 100 mM KCl or 100 mM NaCl and 10 mM Hepes–Tris, pH = 7.4. Vesicles (10 μL) were added to 40 μL extravesicular medium. Values are expressed as pmol/mg protein (mean ± SD).

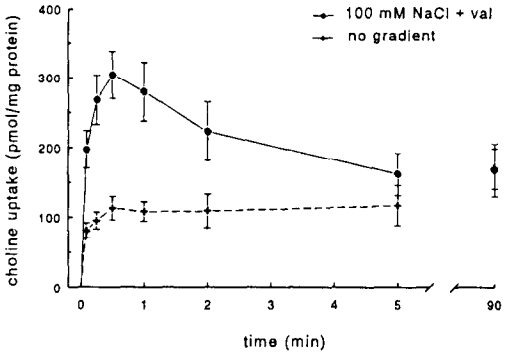

Fig. 2. Effect of an inside negative membrane potential on the uptake of 25 μM choline into SMMV. Vesicles, suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes–Tris, pH = 7.4, were pre-equilibrated with 20 μM val at 37°. Extravesicular media consisted of 100 mM mannitol, 100 mM KCl or 100 mM NaCl and 10 mM Hepes–Tris, pH = 7.4. Vesicles (10 μL) were added to 40 μL extravesicular medium. Values are expressed as pmol/mg protein (mean ± SD).

were frozen in liquid nitrogen and stored at −80° for maximally 4 weeks. The alkaline phosphatase enrichment of SMMV, measured according to Mircheff [11] compared to starting mince was 24-fold higher (M_0 = 60 ± 7 and SMMV = 1400 ± 175 μmol/hr/mg, N = 6). Protein was assayed with a Coomassie blue kit (Biorad, München, Germany).

Uptake studies. Uptake of [^{3}H]choline into SMMV was measured in quadruplicate at 37° using a rapid filtration technique [12]. The samples were filtered through Whatman GF/F filters and the radioactivity remaining on the filters was counted in a Beckman LS 6000 LL liquid scintillation counter. Corrections were made for non-specific filter binding. Exact compositions of the transport conditions are given in the legends. Uptake is expressed as pmol or nmol/mg protein or % of control uptake (mean ± SD) of at least three experiments with three placentas. Paired Student's *t*-test was used to determine statistical significance ($P < 0.05$). Curve fitting was done by least squares non-linear regression analysis using the computer programme PCNONLIN [13].

RESULTS

Time dependent uptake of choline

The effect of an imposed gradient of H^+ (pH_i = 6.0/pH_0 = 7.4) and Na^+ (100 mM) on the uptake of 25 μM [^{3}H]choline into SMMV is illustrated in Fig. 1. An outwardly directed proton gradient did not stimulate the choline uptake (A). Neither did an inwardly directed sodium gradient (B). The effect of an inside negative membrane potential is shown in Fig. 2. A stimulation of the uptake above equilibrium was observed (peak versus equilibrium = 1.8).

Counter transport of choline

The effect of loading the vesicles with 5 mM unlabeled choline on the uptake of 250 μM [^{3}H]-choline into SMMV is shown in Fig. 3. Voltage clamp conditions were maintained by adding val and equal concentrations of intra- and extravesicular K^+. The outwardly directed choline gradient stimulated the choline uptake above equilibrium (peak versus equilibrium = 2.1).

Effect of different drugs on choline uptake

Figure 4 shows the effect of 5 mM of various drugs on the uptake of 250 μM [^{3}H]choline into SMMV, loaded with 5 mM unlabeled choline. Voltage clamp conditions were maintained. The structure analog HC-3, choline itself and the organic cation transport inhibitor mepi reduced choline uptake by 85%. Cationic drugs like cimetidine and famotidine were able to inhibit uptake by 50%. Low molecular mass organic cations like TEA and NMN showed no inhibitory effect, neither did the organic anion transport inhibitor probenecid.

Kinetics of choline uptake

Over a range of 125 μM to 5 mM, saturability in

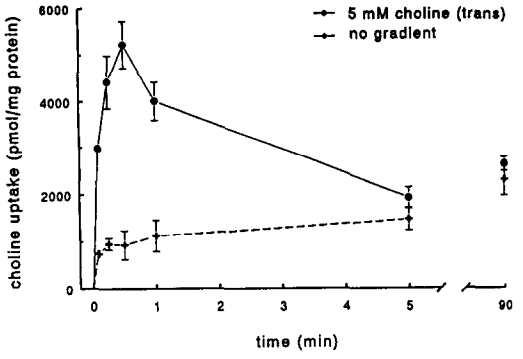

Fig. 3. Effect of an outwardly directed gradient of 5 mM choline on the uptake of 250 μM choline into SMMV. Vesicles, suspended in 100 mM mannitol, 100 mM KCl and 10 mM Hepes–Tris, pH = 7.4, were, in the case of the outwardly directed choline gradient, pre-equilibrated with 5 mM unlabeled choline at 37°. Control and with choline loaded vesicles were also pre-equilibrated with 20 μM val at 37°. Extravesicular media consisted of 100 mM mannitol, 100 mM KCl, 20 μM val, 10 mM Hepes–Tris, pH = 7.4 and unlabeled choline to achieve an extravesicular concentration of choline of 250 μM. Vesicles (5 μL) were added to 195 μL extravesicular medium. Values are expressed as pmol/mg protein (mean ± SD).

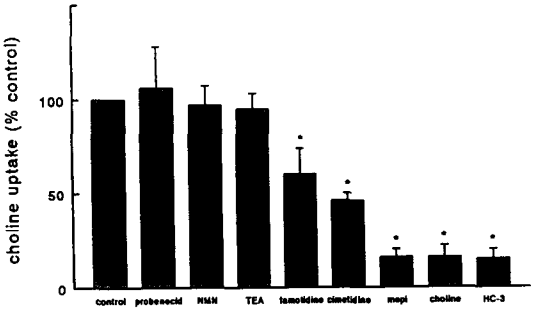

Fig. 4. Effect of 5 mM *cis* concentrations of organic cations and anions on the 10 sec uptake of 250 μM choline into SMMV in the presence of an outwardly directed gradient of 5 mM choline. Experimental conditions were the same as described in the legend of Fig. 3, except that 5 mM of specified organic drugs were added to the extravesicular media. Values are expressed as per cent of control uptake (mean ± SD). Control uptake (without inhibitor) was 3130 ± 515 pmol/mg protein. *Statistical significance ($P < 0.05$; df = 2).

the uptake of [^{3}H]choline was seen in the presence of an outwardly directed 5 mM choline gradient (Fig. 5). Uptake values were measured under voltage clamp conditions. The curve resulting from subtracting simple diffusion (that part of total uptake that could not be inhibited by extravesicular choline) from total uptake was analysed according to Michaelis–Menten kinetics. Least-squares non-linear regression analysis revealed a K_m of 550 ± 80 μM

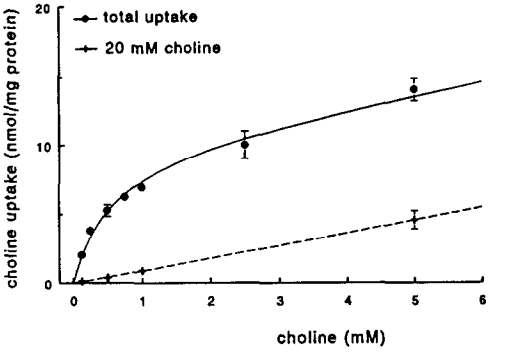

Fig. 5. Concentration dependent uptake of choline at 10 sec into SMMV in the presence of an outwardly directed gradient of 5 mM choline with and without 20 mM choline in the extravesicular medium to saturate the carrier in order to determine the simple diffusion component of total uptake. Experimental conditions were the same as described in legend of Fig. 3. Uptakes were measured in the presence of increasing concentrations choline (0.25–5 mM). Values are expressed as nmol/mg protein (mean ± SD).

and a V_{max} of 10.0 ± 0.49 nmol/mg protein/10 sec ($r^2 = 0.995$).

DISCUSSION

Our results in SMMV, confirm the existence of an uphill mediated transport mechanism for choline in human placenta. A transient accumulation of choline was seen in the presence of an outwardly directed choline gradient, which is considered to be indicative for carrier mediated transport. Uptake into SMMV was also stimulated above equilibrium uptake by an inside negative membrane potential. We conclude that the physiological negative cell interior can act as a driving force for placental choline uptake from the maternal circulation. The uptake into SMMV was sodium independent. This result is in good agreement with the sodium independent uptake into the trophoblast, as was demonstrated in the dually perfused guinea pig placenta [8]. Choline transport in rabbit renal brush-border membranes [7], human erythrocytes [5] and rat intestinal brush-border membranes [6] is also sodium independent in contrast to the uptake in neural tissue, which is sodium coupled [4]. It seems that a sodium gradient, maintained by Na^+/K^+ ATPase, is not directly involved in choline transport in most tissues including the human placenta.

Several cations were unable to inhibit choline uptake at relatively high concentrations. The analog HC-3, choline itself and the organic cation transport inhibitor mepi inhibited the choline uptake almost completely, whereas cationic drugs like cimetidine and famotidine only partly reduced choline uptake. Therapeutic plasma concentrations of these H_2-receptor antagonists are low, which means that the clinical relevance of this inhibition, at concentrations as high as 5 mM, will be limited. NMN and the quaternary ammonium compound TEA did not

inhibit the uptake. In rabbit renal brush-border membranes TEA also had no inhibitory potency [7], whereas in rat intestinal brush-border membranes TEA and NMN caused both *cis*-inhibition and *trans*-stimulation [6]. Due to the strong inhibition of choline and HC-3 as compared to the other cations we suggest a relatively high specificity of the choline transporter in placental SMMV. Whereas our results indicate an affinity of cationic drugs for the choline carrier the reverse, an affinity of choline for the organic cation/proton antiporter for guanidine could not be determined in placental brush-border membranes [14]. In the same study cimetidine did not inhibit this guanidine carrier. It seems that exogenous organic cations interfere with the choline transporter rather than with the guanidine transporter.

The kinetics determined in SMMV involved a saturable process with a K_m of 550 μM, corresponding to a value of 350 μM found in placental fragments [2] and greater than 97 and 159 μM found in other epithelia like rabbit renal brush-border membranes [7] and rat intestinal brush-border membranes [6], respectively. A K_m of 550 μM, which is far above the maternal plasma concentration of 20 μM, assures an efficient uptake of choline into the trophoblast.

In conclusion, the transport system for choline in SMMV of human term placenta demonstrated here, can efficiently provide for the placental uptake of choline from the maternal circulation driven by the negative cell interior. It is believed that this syncytial accumulation is essential for the permeability of the trophoblast for amino acids [3] or for the fetal need for choline. The apparent discrepancy between the low placental transfer as shown by Sweiry *et al.* [9] and the significant placental uptake of choline suggests that the placenta will function as a buffer for the fetal need of choline. Fetal choline uptake will therefore be regulated mainly by the transfer across the basal membrane of the trophoblast. Mechanisms of choline uptake across the basal membrane of the human placental trophoblast are currently under investigation.

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REFERENCES

1. Welsch F, Choline metabolism in human term placenta—studies on *de novo* synthesis and the effect of some drugs on the metabolic fate of [N-Me-^{3}H] choline. *Biochem Pharmacol* **27**: 1251–1257, 1978.
2. Welsch F, Studies on accumulation and metabolic fate of [N-Me-^{3}H] choline in human term placental fragments. *Biochem Pharmacol* **25**: 1021–1030, 1976.
3. Rowell PP and Sastry BVR, Human placental cholinergic system: depression of the uptake of α-aminoisobutyric acid in isolated human placental villi by choline acetyltransferase inhibitors. *J Pharmacol Exp Ther* **216**: 232–238, 1981.
4. Kuhar MJ and Murin LC, Sodium dependent, high affinity choline uptake. *J Neurochem* **30**: 15–21, 1978.
5. Deves R and Krupka RM, The binding and translocation steps in transport as related to substrate structure; a study of choline carrier of erythrocytes. *Biochim Biophys Acta* **557**: 468–485, 1979.
6. Saitoh H, Kobayashi M, Sugawara M, Iseki K and Miyazaki, Carrier mediated transport system for choline and its related quaternary ammonium compounds on rat intestinal brush-border membrane. *Biochim Biophys Acta* **1112**: 153–160, 1992.
7. Wright SH, Wunz TM and Wunz TP, A choline transporter in renal brush-border membrane vesicles: energetics and structural specificity. *J Membr Biol* **126**: 51–65, 1992.
8. Sweiry JH and Yudilevich DL, Characterization of choline transport at maternal and fetal interfaces of the perfused guinea-pig placenta. *J Physiol* **366**: 251–266, 1985.
9. Sweiry JH, Page KR, Dacke CG, Abramovich DR and Yudilevich DL, Evidence of saturable uptake mechanisms at maternal and fetal sides of the perfused human placenta by rapid paired-tracer dilution: studies with calcium and choline. *J Dev Physiol* **8**: 435–445, 1986.
10. Glazier JD, Jones CJP and Sibley CP, Purification and Na^+ uptake by human placental microvillous membrane vesicles prepared by three different methods. *Biochim Biophys Acta* **945**: 127–134, 1988.
11. Mircheff AK and Wright EM, Analytical isolation of plasma membranes of intestinal epithelial cells: identification of Na^+/K^+-ATPase rich membranes and the distribution of enzyme activities. *J Membr Biol* **28**: 309–337, 1976.
12. Russel FGM, van der Linden PEM, Vermeulen WG, Heijn M, van Os CH and van Ginneken CAM, Na^+ and H^+ gradient dependent transport of *p*-aminohippurate in membrane vesicles from dog kidney cortex. *Biochem Pharmacol* **37**: 2639–2649, 1988.
13. Metzler CM and Weiner DL, PCNONLIN (3.0) *Software for the Statistical Analysis of Nonlinear Models on Micros*. SCI Software, Lexington, U.S.A., 1989.
14. Ganapathy V, Ganapathy ME, Cherukantath NN, Mahesh VB and Leibach FH, Evidence for an organic cation-proton antiport system in brush-border membranes isolated from the human term placenta. *J Biol Chem* **263**: 4561–4568, 1988.