Biotin Uptake by Basolateral Membrane Vesicles of Human Placenta: Normal Characteristics and Role of Ethanol (43778)

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Abstract. **This study assessed the mechanism of uptake of biotin by the fetal-facing (basolateral) membrane of the term human placenta. Using membrane vesicles, we showed that most of the uptake was attributable to transfer of the vitamin into the vesicle and that the uptake was saturable, Na-dependent, carrier-mediated, and electroneutral. The rate of uptake was less than for biotin uptake by the maternal-facing (apical) membrane of the human placenta. Because ethanol inhibits biotin uptake by the apical membrane, the effect of ethanol on uptake by basolateral vesicles was investigated. With 10-hr exposure at a concentration of 2 and 3 mg/ml, but not 1 mg/ml, ethanol modestly inhibited biotin uptake. The mechanism of inhibition by alcohol is not known.** [P.S.E.B.M. 1994, Vol 206]

Biotin is a water-soluble vitamin; it serves as an essential coenzyme for four mammalian carboxylases which catalyze several important reactions in metabolic pathways (1). Biotin thus participates in growth and development of tissues; biotin deficiency causes fetal growth abnormalities (2, 3).

Fetal biotin is derived solely from the mother by transplacental transfer. We, and others, have recently characterized biotin transport by the term human placenta (4, 5). Studies using maternal-facing (apical) placental vesicles showed biotin uptake to be carrier-mediated and energy-dependent, while overall maternal to fetal transfer across the placenta (using the isolated perfused cotyledon model) was passive (4, 5). A key component in this transport process might be the fetal-facing (basolateral) membrane, but this transport process has not yet been studied. We report here studies of the mechanism of uptake of biotin by the basolateral membrane of the human term placenta. Because ethanol consumption in pregnancy may lead to fetal growth abnormalities (6) and because ethanol was shown in our earlier studies to impair the uptake of biotin by the apical placental membrane (4), the effect of ethanol on uptake of biotin by the basolateral membrane was now also studied.

Materials and Methods

Fetal-facing (basolateral) vesicles were prepared from freshly obtained term normal human placenta by the procedure of Dicke, *et al.* (7). Briefly, a 15-g portion of placenta is passed through a filtration mesh into 50 m*M* Tris-HCl (pH 7.4) at 4°C. After sonication, filtering, and washing to remove the apical membrane, the residual tissue is incubated in 10 m*M* EDTA adjusted to isotonicity by addition of 200 m*M* sucrose, 40 m*M* Tris-HCl, and 3 m*M* Tris base (7). Separation of the basal cell membrane from the basal lamina is accomplished by a second incubation and sonication, after which the tissue is removed by filtration through multiple layers of gauze. Large cell contamination is removed by centrifugation at 4,000*g* for 10 min then at 10,000*g* for 20 min. The resulting supernatant is centrifuged at 90,000*g* for 40 min yielding a preparation of

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basal membrane of the syncytiotrophoblast. Final purification is accomplished by resuspending the pellets in Tris-sucrose buffer. The vesicles are quick frozen and stored at −70°C prior to use. These membrane preparations showed a 20-fold enrichment of dihydroalprenolol binding (basolateral marker enzyme) and a 4-fold enhancement (versus homogenate) of the apical membrane marker, alkaline phosphatase. This degree of purity is consistent with the data of others for this membrane in human placenta (8). Immediately before individual studies, the vesicles are thawed. To make a 100 m*M* Na^+ gradient between intra- and extra-vesicular space, vesicles in Tris-sucrose buffer are subjected to a final centrifugation at 10,000*g* for 40 min. The pellets are then resuspended in HTM-300 (HEPES 10 m*M*, Tris 10 m*M*, and Mannitol 300 m*M*, adjusted with acid or base to pH 7.4). Transport reaction is carried out in HTM-100 incubation buffer which is composed of HEPES to m*M*, Tris 10 m*M*, Mannitol 100 m*M*, and NaCl 100 m*M*.

^{3}H-biotin (purity 99.9%, specific activity 1.7 TBq/mmol) was obtained from Dupont (NEN). A concentration of 5 n*M* was used in most studies; this concentration approximates the physiologic concentration of free biotin in serum (approximately 1 n*M*).

Determinations of the uptake of labeled biotin were carried out by adding an aliquot of microvesicle suspension (20 μl containing 0.2 mg protein) to 170 μl of incubation buffer. After mixing with buffer, the biotin is added in 10 μl of buffer and mixed again. After selected incubation times at 25°C, usually 45 sec, the transport (other than diffusion) is stopped by adding 3 ml of ice cold buffer. The mixture is then immediately filtered through HAWP filters of 0.45 micron-pore size. Five 3-ml washes remove all unbound label. The filter is then dissolved in 1 ml ethylene glycol and the radioactivity taken as amount bound. This is expressed per μg of vesicle protein which is determined by the procedure of Lowry, *et al.* (9).

Some experiments were done with 100 m*M* Na^+ gradient; in others, sodium was substituted by equimolar K^+. To characterize the transport mechanism, incubations were carried out at various temperatures with addition of 100-fold greater concentrations of putative structural antagonists or with ethanol (1, 2, or 3 mg/ml) for 10 hr. To assess the role of electrogenic effects in transport, in some studies gramicidin or valinomycin was added to the incubate exactly as described by Gassl (15). Membrane vesicles were preincubated with either 0.25 mg/ml gramicidin or valinomycin for 30 or 60 min. All vesicles were preloaded with 50 m*M* K^+ and incubated in HTM-100 buffer. The 45 sec total 5 n*M* biotin uptake in 100 m*M* Na was compared in the absence or presence of gramicidin or valinomycin. For experiments assessing biotin metabolism, the reaction was stopped by the addition of 1.8 ml of ice-cold 0.05% trifluroacetic acid, pH 2.5 instead of buffer. The resulting 2.0 ml volume was filtered through a 0.45 micron filter to remove vesicles and particulates. A 1.0 ml sample was analyzed by HPLC and metabolism of biotin was assessed by HPLC as previously reported (4, 10).

Statistical analysis was carried out by the Student's *t* test with *P* values of <0.05 (two-tailed) taken as statistically significant. However, for assessment of the ethanol effect on biotin transport, where multiple concentrations of ethanol were used, we employed ANOVA with Dunnett's multiple comparison procedure and the Bonferroni multiple comparison analysis.

Results

As is shown in Figure 1, there was an inverse and linear relationship between the concentration of mannitol in the incubate (and presumably medium osmolarity) and the uptake of biotin by the vesicles. Thus, as osmolarity decreased, the vesicular space and biotin uptake increased, suggesting that the vitamin was being primarily transported into the vesicle rather than binding to it. However, the uptake line intersects the *y* axis above zero, suggesting that some biotin is bound to the membrane. From the *y* axis intercept, it could be calculated (*y* − intercept/uptake at 300 m OSM) × 100

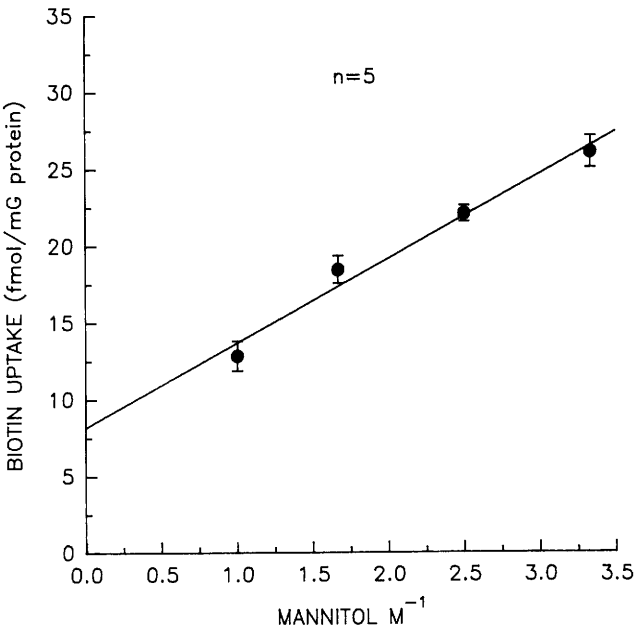

Figure 1. Effect of medium osmolarity on biotin uptake. A 20-μl quantity of basal membrane preparation (200 μg protein) was incubated with a 170-μl quantity of mannitol buffer of the specified osmolarity for 30 min. Ten microliters of 0.1 μ*M* biotin were added to the vesicles and the reaction stopped after 45 sec. Each point is the mean ± SE of five separate assays. As the mannitol concentration and osmolarity decreased, vesicular space and biotin uptake increased suggesting primarily uptake *into* the vesicle ($r = 0.943$). As the positive intercept on the *y* axis implies a smaller portion of the biotin (see Results) was *bound* to the membrane.

that 69.1% of biotin uptake under iso-osmolar conditions is due to its transfer into the vesicle and the rest can be attributed to binding. Small binding by basolateral vesicles from rat and human liver has been reported earlier (11, 12).

As shown in Figure 2, there was an essentially linear uptake of the vitamin for 45 sec with 5 n*M* biotin; thereafter a distinct "overshoot" phenomenon was observed (13). Total uptake of biotin was clearly dependent on a Na^+ gradient and temperature (Figs. 2 and 3), and was saturable and dependent on the presence of sodium (Fig. 4). The calculated V_{max} was 43.5 femtomol/min/mg protein, and the K_m was 5.71 n*M*. The saturable total transport of biotin was inhibited substantially by three structural analogues of biotin: desthiobiotin, biocytin, and biotin methylester at concentrations 100-fold greater than that of biotin (Fig. 5). These composite data are compatible with a carrier-mediated, Na^+-dependent, active uptake of biotin by the basolateral membranes. To determine if this process is electrogenic in nature, we assessed the effects of gramicidin or valinomycin (altering the electrical potential across the membrane) on biotin uptake. With both 30- and 60-min preincubation with gramicidin and valinomycin there was no effect on biotin uptake, implying absence of electrogenic biotin uptake. We also assessed the metabolism of ^{3}H-biotin by the basolateral membrane as described earlier. Binding of ^{3}H-biotin to the microvesicles and metabolism was directly measured after addition of hypotonic, acidic buffer to halt metabolism and rupture the vesicles as described in Materials and Methods. Binding was less than 5% and we detected less than 1% metabolism of biotin to any other analog.

We next assessed the effects of three concentrations of ethanol (1, 2, and 3 mg/ml for 10 hr) on biotin

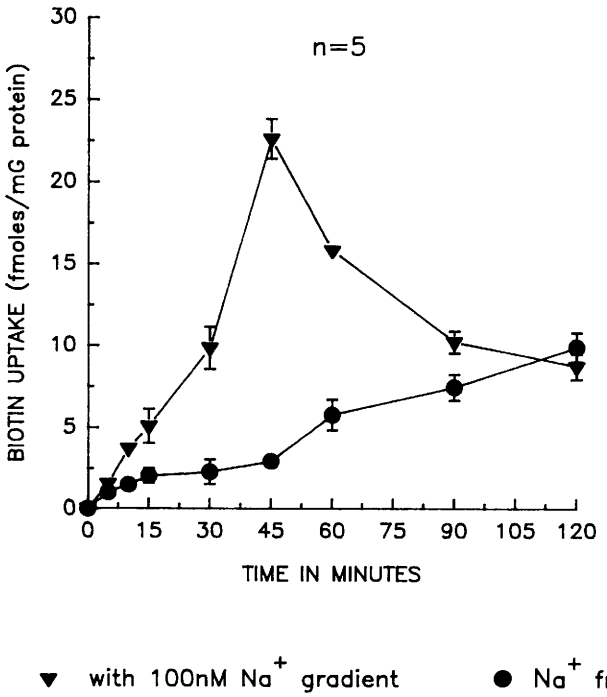

Figure 2. Uptake of biotin by the basolateral placental membrane. Uptake with time of 5 n*M* ^{3}H-biotin (specific activity 1.7 TBq/mmol) in the presence and absence of 100 m*M* Na^+ is shown. 100 n*M* K^+ was substituted for Na^+, where required. There is evidence of linearity to about 45 sec with an "overshoot" phenomenon peaking at that time. Each point is the mean ± SE of five assays in separate vesicle preparations.

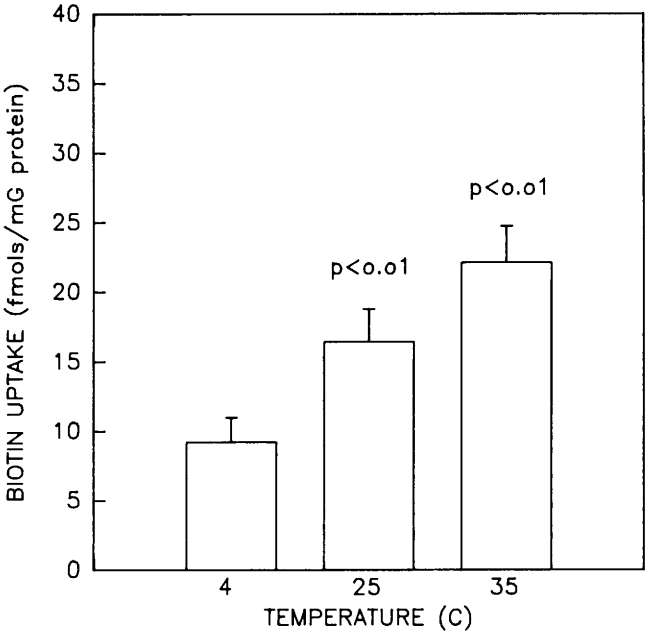

Figure 3. Temperature-dependence of biotin uptake. 5 n*M* total biotin uptake at 45 sec for three different incubation temperatures is shown. Each bar is the mean ± SE of six studies. The increase in uptake with increasing temperature is evident.

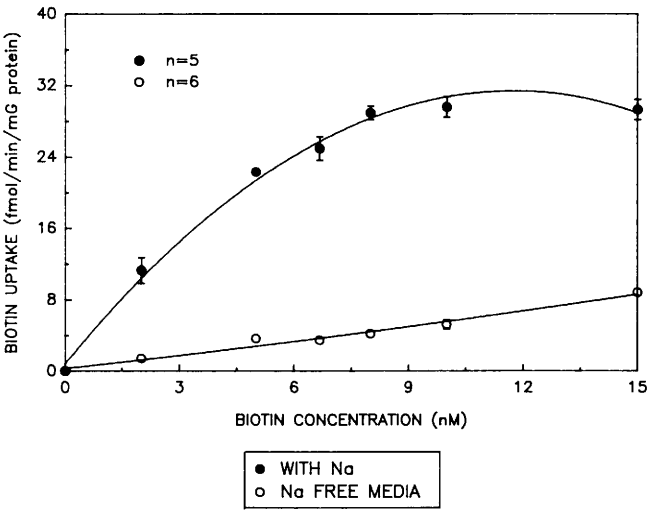

Figure 4. The effect of sodium on biotin uptake. Uptake of biotin (varying concentrations) at 45 sec with and without 100 n*M* Na^+ is shown. In the absence of Na^+, equimolar K^+ was used. Each point is the mean ± SE of five to six studies. Evidence of saturation kinetics in the presence of Na^+ and the more rapid uptake with Na^+ are evident.

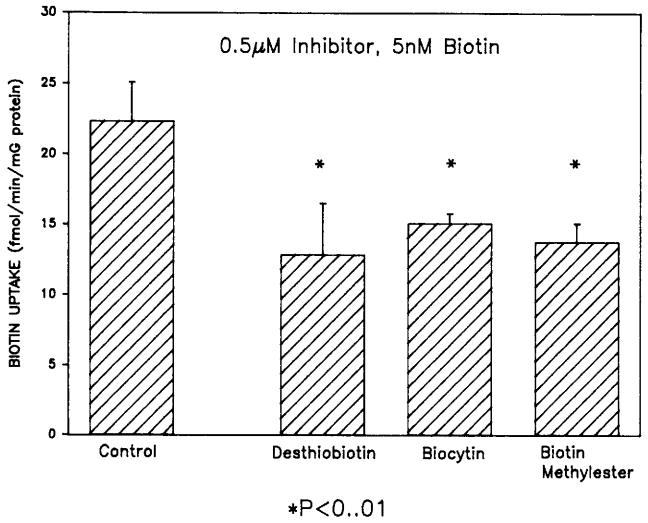

Figure 5. Effect of structural antagonists of biotin on biotin uptake. Total biotin (5 n*M*) uptake in 100 m*M* Na^+ at 45 sec was studied in the absence of structural inhibitors and with 100-fold higher concentration of desthiobiotin, biocytin, or biotin methylester. Each bar is the mean ± SE of eight studies. The statistically significant inhibition of biotin uptake with each antagonist is shown.

uptake in the presence of 100 m*M* Na^+. As shown in Figure 6, 1 mg/ml ethanol had no significant effect on biotin uptake. Both 2 and 3 mg/ml ethanol decreased

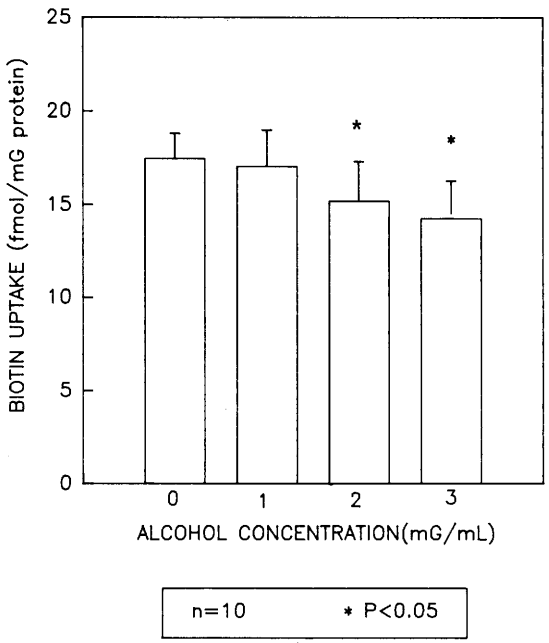

Figure 6. Effect of varying concentrations of ethanol on the uptake of biotin. Total biotin (5 n*M*) uptake in the presence of 100 m*M* Na^+ at 45 sec was studied in the absence of ethanol and after exposure to 1, 2, or 3 mg/ml ethanol for 10 hr. Each bar is the mean ± SE of 10 studies. There was no statistically significant effect of 1 mg/ml ethanol, while 2 and 3 mg/ml ethanol significantly, albeit modestly, lowered biotin uptake. The data were significantly different from control at the $P < 0.05$ by Dunnett's test and at the $P < 0.016$ by Bonferroni's multiple comparison test.

biotin transport slightly. The decrease in biotin uptake at 3 mg ethanol/ml, however, was only 18.4%. In the absence of Na^+ (data not shown), exposure to 3 mg/ml ethanol for 10 hr ($n = 10$) decreased biotin uptake by only 6.8% ($P = 0.054$).

Discussion

This study provides strong evidence that the uptake (and presumably transfer) of biotin by the fetal-facing basolateral membrane of the normal term human placenta is saturable, carrier-mediated, Na^+-dependent, and electroneutral, and that transport also appears to require energy. We believe this is the first characterization of biotin transport by the basolateral placental membrane. Transport by the basolateral membranes of rat and human liver and rat intestine has been studied; each system is reported to be carrier-mediated and Na^+-dependent (11–13). With the exception of rat liver (11), the basolateral transport has also been shown to be electroneutral. In the apical human placental membrane, there is some conflict whether biotin transfer is electrogenic (15) or not dependent on membrane potential (5).

Biotin uptake by the maternal-facing (apical) membrane is also reported to be carrier-mediated and Na^+-dependent (4, 5); however, the studies reported here indicate that the basolateral membrane transport is considerably (at least 10-fold) slower than apical transport. Interestingly, overall transfer of biotin by the human placenta, from maternal to fetal circuits, using the single, isolated, perfused cotyledon system, is passive (4, 5). One interpretation of this observation is that the transport of biotin through the cytoplasm between the apical and basolateral membranes is rate-limiting. The mechanism leading to a rate-limiting cytoplasmic transport is not known. Binding of biotin in the perfusate (or serum) is only modest. Binding to placental tissue and the attendant concentration of biotin in placental tissue is not great (4). Further, no metabolism of biotin was seen during isolated placental perfusion for 4 hr (4, 5); hence, metabolism to a tightly bound metabolite is not likely to be important during uptake studies using only a membrane component and lasting, at most, several minutes. Indeed, no such metabolite was identified in this study with basolateral vesicles. In the osmolarity studies, up to 30% of the biotin was found to bind to the vesicle; however, in the metabolism studies, less than 5% bound. We speculate that this difference may be attributed to release of bound biotin by the addition of hypotonic, acidic buffer prior to measuring biotin in the metabolic studies.

As in the apical membrane (4), ethanol at 2- and especially at 3-mg/ml concentrations modestly inhibited biotin uptake by the basolateral membrane. In

both membranes, a lower concentration of ethanol did not alter biotin uptake. The mechanism(s) of this effect of ethanol is uncertain. It is interesting, however, that ethanol impaired carrier-mediated biotin uptake by both membranes even though the overall transfer by the placenta was observed to be a passive process that was not altered by exposure to alcohol (4). We speculate that, on biotin transported in the basolateral membrane, the observed effect of ethanol could theoretically be mediated by changes in Na^+,K^+-mediated ATPase (16, 17), as suggested by us earlier for alcohol-induced impairment of thiamine transport by the intestinal basolateral membrane (18). This hypothesis is consistent with the observation that ethanol has less pronounced effects on Na^+-independent biotin uptake than on Na^+-dependent biotin uptake; however, this hypothesis does not explain the alcohol-induced decrease in apical biotin uptake (4). Possibly, ethanol exposure has an effect on the physical properties of these membranes. Further mechanistic studies of the effects of ethanol on biotin transport in placental apical and basolateral tissue are needed.

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