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Transport Kinetics of Alpha-Aminoisobutyric Acid in the Perfused Human Placental Lobule in vitro

Abstract

Objective: The transports kinetics of α -aminoisobutyric acid (AIB) has been investigated in the human placenta in vitro using perfusion of isolated placental lobules. **Methods:** NCTC 135 tissue culture medium diluted with Earle's salt solution was used as the perfusate, and tritiated water was used as the reference marker. **Results:** The transport fraction of AIB averaged 13.2% of that of tritiated water in 5 single-bolus injection experiments. The differential transport rates for 10, 25, 50, 75 and 90% cumulative efflux in the fetal vein of the study and the reference substances differed significantly as well. The transport kinetic parameters as area under the curve, clearance, elimination constant, time for maximum response, absorption rate and elimination rate of the amino acid averaged 0.12, 19.8, 0.78, 1.28, 0.10 and 0.06 times, respectively. Plotting of relative AIB transport ratios as a function of perfusion time demonstrated the active nature of amino acid transport in our perfusion conditions. **Conclusions:** The transport and kinetic parameters employed in the study may be useful in assessing dynamic placental membrane function of A type amino acids and other substances in varying experimental conditions as well as in disease states.

Key Words

Transport kinetics
 α -Aminoisobutyric acid
Human placenta
In vitro
Tritiated water
Maternal-fetal exchange

Introduction

α -Aminoisobutyric acid (AIB) has been widely used as a marker for placental permeation studies, both in humans [1, 2] as well as in animals [3, 4]. Its nonmetabolizable nature

has permitted the investigators to specifically explore amino acid transport independent of maternal and fetal metabolic influences. Transport studies of this amino acid in humans have centered mainly on in vitro perfusion of isolated placental lobules, focusing on

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the steady state rather than the dynamic state of membrane function. Hence, we thought it interesting to explore the transport kinetics of AIB in the perfused placental tissue, using a modified experimental protocol and using specifically designed transport parameters. The bolus injection method was chosen when compared to the steady-state perfusion method, since the former was found to be more suitable than the latter to assess dynamic transport function characteristics of the substances in question. Previous studies in our laboratory [5, 6] had established that such a protocol served to delineate transport kinetics of study substances independent of minor variations in factors such as perfusion flow rate, dosage or membrane surface area. Tritiated water was used as the reference because of its flow-limited characteristics. Further, its use has permitted us to minimize possible experimental artifacts as physiological shunts or changes in the membrane surface area and to obtain control data, permitting comparison with similar studies in varying experimental or disease situations.

Materials and Methods

Human placentae were collected post partum after uncomplicated pregnancies, and perfusion of suitable placental lobules was performed as previously described [7–10]. Briefly, fetal vessels of an isolated placental lobule were cannulated and perfused with NCTC 135 (Sigma Chem. Co., St. Louis, Mo., USA) tissue culture medium – Earle's solution mixture [2] being the perfusate. Bovine serum albumin at a concentration of 1 g/l was added to the perfusate to compensate for the absence of plasma proteins in the perfusion medium. Perfusion was effected using a Harvard digital pump. Flow rates of the perfusate in the fetal and maternal circuits were monitored by flow meters (Brooks R 215 A), and perfusion pressure was monitored by mercury manometers. The viability of the preparation was assessed by determining the oxygen and glucose consumption and the CO₂ production of the tissue during perfusion.

Experimental Design

After an initial washout period of 10 min, 1 µCi of tritiated water (specific activity: 5 mCi/mmol, Amersham, UK) and 0.5 µCi of ¹⁴C-AIB (specific activity: 60 mCi/mmol, Amersham) were injected as 100-µl bolus into the maternal circulation at a site close to the insertion of the microcannulas in the basal plate. After a period of 1 min, serial perfusate samples were collected from the fetal venous outflow every 30 s for a period of 5 min. The time lag of 1 min before the start of the sample collection was determined in previous control experiments, by injecting a 100-µl bolus of Indian ink into the maternal circulation at the preselected site and by noting the time interval for the dye to first appear on the basal plate. The effective study period of 5 min was fixed after noting the time required for 90% of the injected radioactivity to appear in the combined fetal and maternal venous effluents in the control experiments, after taking into account the 1-min lag period mentioned earlier.

Radioactivity (dpm) in the different perfusate samples and injected boluses was determined by liquid scintillation counting (LKB Wallac model). Necessary precautions were taken to minimize errors from quenching and isotope crossover in counts, by adjusting the tritium and ¹⁴C windows and by using standards with known radioactivity concentrations.

Transport Parameters

(1) The maternal-fetal transport of AIB and tritiated water was expressed as differential transport rate (TR), expressed as time in minutes for a given fraction to be transported across to the fetal vein [5].

Efflux fraction =

$$\frac{{}^3\text{H or } {}^{14}\text{C dpm in fetal venous sample}}{\text{total } {}^3\text{H or } {}^{14}\text{C dpm in fetal vein for a period of 5 min}}$$

From the curve obtained by plotting various cumulative efflux fractions as a function of perfusion time, the time in minutes for an efflux of 10, 25, 50, 75 and 90% of total venous efflux was then computed. Control studies had established that these efflux times, 6 for the study substances, varied little with increasing or decreasing doses for a given set of fetomaternal flow rates.

(2) The transport rate index (TI) of the test substance was then computed for 50% of the efflux by the following formula:

$$\text{TI (AIB)} = \frac{\text{AIB TR}_{50}}{\text{H}_2\text{O TR}_{50}}$$

(3) The transport fraction (TF) of the studied substances was calculated thus:

TF =

$$\frac{\text{total } ^3\text{H or } ^{14}\text{C dpm in fetal venous efflux for a period of 5 min}}{\text{total } ^3\text{H or } ^{14}\text{C dpm in the injected bolus}}$$

Calculation of the ratio relating to the TF of the test studied substance to that of the reference substance will permit assessment of the TI of the former, independent of minor variations in flow rate, shunts or changes in membrane surface area.

(4) The area under the curve (AUC) of the studied substances was determined by trapezoid rule, assuming a two-compartment model and using the following formula [5, 6, 11]:

$$\text{AUC} = \sum_{i=1}^n \frac{[C(i+1) + C(i)] \times [t(i+1) - t(i)]}{2} + \frac{C(n)}{K_{el}};$$

where C = concentration of the substance in the sample, t = time in seconds of sample collection, n = number of experimental points, C_n = last experimental point or measurement and K_{el} = elimination constant.

Other kinetic parameters as clearance, K_{el}, T_{max} (time for maximal response), absorption rate and elimination rate were determined using a computer program based on IMSL Fortran Subroutine software, specially adapted for statistical applications. The software was run on a VAX/VMS 6510 computer.

(5) To assess the active or passive nature of AIB transport, another transport parameter was also employed. The ratio of AIB label counts to tritiated water label counts was computed in the different perfusate samples in the fetal vein, and the ratios were plotted as a function of perfusion time for the whole duration of the experiment. It was premised that the ratio of the two labels in the injectate being constant, any increase in the relative AIB label ratio in the fetal venous effluent as a function of perfusion time would be indicative of the active nature of transport of the amino acid.

Results

Figure 1 shows the efflux pattern of tritiated water and AIB in a typical perfusion experiment. This pattern was observed in all 5 successful perfusions. Table 1 summarizes the differential transport rates of tritiated water and AIB for 10, 25, 50, 75 and 90% efflux in

the fetal vein. The AIB TR averaged 0.36 ± 0.14 , 0.77 ± 0.11 , 1.44 ± 0.12 , 2.81 ± 0.14 and 3.52 ± 0.17 min, respectively, for 10, 25, 50, 75 and 90% efflux fractions in the 5 perfusions, representing 1.61 ± 0.08 , 1.59 ± 0.11 , 1.84 ± 0.09 , 1.62 ± 0.18 and 1.14 ± 0.16 times those of tritiated water values. The paired Student's t test showed that the TR of both substances differed significantly ($p < 0.05$) for all the 5 efflux points investigated. The TI of AIB expressed as (TR₅₀ AIB)/(TR₅₀ H₂O) ratio, averaged 1.84 ± 0.09 in the above experiments.

The fetal perfusate flow rate averaged 4.4 ± 0.3 ml/min while the maternal flow rate averaged 10.6 ± 0.4 ml/min in the 4 successful perfusions. The cotyledon weight averaged 28.6 ± 2.4 g.

The TF of AIB averaged $0.22 \pm 0.08\%$ of the injected amino acid dose in the 5 perfusions, while that of tritiated water averaged $1.48 \pm 0.07\%$. Student's t test showed that the TF of AIB differed significantly from that of water ($p < 0.05$). The TF values of AIB represented $13.2 \pm 1.8\%$ of the tritiated water TF in the 5 experiments.

Various transport kinetic parameters of study and reference substances in the 5 perfusions are shown in table 2. When the 2 groups of data were subjected to the paired Student's t test, AUC, clearance, T_{max}, absorption rate and elimination rate were shown to be significantly different ($p < 0.05$), while no significance could be shown ($p > 0.05$) between the 2 groups in the case of K_{el}, because of the inter-experimental variability. AIB kinetic parameters such as AUC, clearance, K_{el}, T_{max}, absorption rate and elimination rate values averaged $56,185 \pm 2,626$ dpm, 45.3 ± 13.8 ml/min, 3.06 ± 0.84 , 124.19 ± 6.9 s, 87 ± 29 dpm and 172 ± 56 dpm, respectively, representing 0.12 ± 0.02 , 19.8 ± 2.2 , 0.78 ± 0.14 , 1.28 ± 0.06 , 0.10 ± 0.02 and 0.06 ± 0.01 times those of the corresponding tritiated wa-

Fig. 1. Efflux pattern in the fetal vein of ¹⁴C-AIB and tritiated water in a typical perfusion experiment.

Table 1. Differential TR of AIB and tritiated water in normal placentae

Substance	Differential TR, min				
	TR ₁₀	TR ₂₅	TR ₅₀	TR ₇₅	TR ₉₀
Tritiated water	0.21 ± 0.07	0.46 ± 0.12	0.67 ± 0.14	1.64 ± 0.15	2.84 ± 0.17
AIB	0.36 ± 0.14	0.77 ± 0.11	1.44 ± 0.12	2.81 ± 0.14	3.52 ± 0.17

Values represent means + SEM of 5 successful perfusions.

ter values. Relative AIB TR, plotted as a function of perfusion time, is shown in figure 2. A strong correlation was shown between the 2 groups ($r^2 = 0.972$) when the data were subjected to linear-regression analysis by the least-square method.

Discussion

The results on tritiated water transport kinetics are consistent with earlier reports [5, 6] of our research group. The data also fit well with those of international research groups

Fig. 2. Relative TR of AIB in different perfusate samples, expressed in relation to corresponding tritiated water label counts. The results are expressed as means of 5 successful perfusions. The linear regression line was drawn using the equation: $y = 3.6365 \times 10^{-3} + 3.8884 \times 10^{-4}x$, $r^2 = 0.972$.

Table 2. Kinetic parameters of AIB and tritiated water in normal placentae

Substance	AUC, dpm	Clearance, ml/min	K_{el}	T_{max} , s	Absorption rate, dpm	Elimination rate, dpm
Tritiated water	$1,143,622 \pm 79,612$	2.66 ± 0.92	5.11 ± 0.71	95.8 ± 6.3	969 ± 98	$3,702 \pm 198$
AIB	$56,185 \pm 2,626$	45.3 ± 13.8	3.06 ± 0.84	124.1 ± 6.9	87 ± 29	172 ± 56

Values represent means $\pm$ SEM of 5 successful perfusions.

demonstrating free placental permeability for water across the placental membrane in animals [3, 12] as well as in humans [10, 13]. Active transport of AIB across the human placenta has already been firmly established [14–16]. Our data on the amino acid transfer are consistent with previous reports on AIB transport in the perfused placental lobule [1, 2, 17]. The AIB TF value of about $0.22 \pm 0.08\%$ compares favorably with that computed from the data of Contractor and Stannard [1] for a comparable duration. By plotting relative AIB

TR in different perfusate samples as a function of perfusion time, and by determining the correlation between the two variables, we were able to confirm the active nature of AIB transport reported by others. Besides, calculation of the TR constant of the amino acid in the human placenta in vitro under controlled experimental conditions will provide the investigators with a useful tool to investigate amino acid transport function in a wide variety of experimental conditions as well as in pregnancy disease states.

Expression of the results as percentage of the cumulative efflux in the fetal vein and plotting of data as function of perfusion time have permitted us to delineate the functional characteristics of the human placental membrane. Control studies have shown that the efflux curve pattern as well as the differential TR of the test substances remained unchanged with regard to the injected dose as well as the perfusion flow rates when minor alterations were introduced in the protocol. However, it was observed in the control studies that wide variations in the flow rates brought about appreciable changes in the pattern of efflux, resulting in wide variations in the differential TR. For this reason, for a given study, the flow rates were kept relatively constant in both the fetal as well as the maternal circuits. A similar variation, however, was not evident when the injected dose was doubled and tripled for a given set of flow rates. Computation of kinetic parameters as AUC, clearance, K_{el} , T_{max} , absorption rate and elimination rate, and expression of the results as ratio of the corresponding tritiated water values have also helped us to delineate membrane characteristics with respect to AIB transport behavior under the specified study conditions. The AUC index value of the ami-

no acid ($12 \pm 2\%$) was not significantly different from the TF index of $13.2 \pm 1.8\%$. Interestingly, the AUC index as well as the TF index of the amino acid compare well with its absorption rate index expressed in relation to that of the reference marker. It is logical to assume that parameters such as the TR_{50} index, relative TR and transport kinetic indices are valuable tools permitting assessment of the dynamic transport function of the placental membrane for a given set of study substances and reference markers in varying experimental conditions as well as in various disease states. This parameter can also be usefully employed to assess transport behavior of A type amino acids [18] in varying experimental conditions. Currently, studies are in progress to evaluate possible alterations in the dynamic placental membrane function with regard to major metabolic fuels in various pregnancy-related disease states.

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