Developmental Changes in Glutamine Transport by Rat Jejunal Basolateral Membrane Vesicles (43076)

FADHEELA T. AL-MAHROOS, NAJI ABUMRAD, AND FAYEZ K. GHISHAN[1]

Department of Pediatrics, Molecular Physiology, Biophysics and Surgery, Vanderbilt University, Nashville, Tennessee 37232

Abstract. **The ontogeny of glutamine uptake by jejunal basolateral membrane vesicles (BLMV) was studied in suckling and weanling rats and the results were compared with adult rats. Glutamine uptake was found to represent a transport into an osmotically active space and not mere binding to the membrane surface. Temperature dependency indicated a carrier-mediated process with optimal pH of 7.0. Transport of glutamine was Na^+ (out > in) gradient dependent with a distinct "overshoot" phenomenon. The magnitude of the overshoot was higher in suckling compared with weanling rats.**

The uptake kinetics and inhibition profile indicated the existence of two major transport pathways. A Na^+-dependent system correlated with System A showed tolerance to System N and System ASC substrates, and a Na^+-independent system similar to the classical L system that favors leucine and BCH.

The V_{max} for the Na^+-dependent system was higher in suckling compared with weanling and adult rats. The V_{max} for the Na^+-dependent system was 0.86 ± 0.17, 0.64 ± 0.8, and 0.41 ± 0.9 nmol·mg protein^{-1}·10 sec^{-1} for suckling, weanling, and adult rats, respectively. The V_{max} for the Na^+-independent system was 0.68 ± 0.08, 0.50 ± 0.03, and 0.24 ± 0.03 nmol·mg protein^{-1}·10 sec^{-1} for suckling, weanling, and adult rats, respectively.

We conclude that glutamine uptake undergoes developmental changes consistent with more activity and/or number of glutamine transporters during periods of active cellular proliferation and differentiation. [P.S.E.B.M. 1990, Vol 194]

Glutamine is the major oxidative substrate of the intestine (1, 2) and is a key amino acid for the synthesis of numerous biologically important compounds and is a carrier form of ammonia (3, 4).

In the developing intestine, there is over 100% protein turnover daily (5) with corresponding high rates of glutamine metabolism (6). This high rate of glutamine metabolism is especially evident during the suckling period that is characterized by rapid growth and development (7, 8). Because the basolateral membranes of the enterocytes provide a major route for glutamine entry, we designed the current studies to define and characterize the developmental changes in glutamine uptake by the jejunal basolateral membranes.

Materials and Methods

Materials. L-[G-^{3}H]glutamine (39 Ci/mmol) was purchased from Amersham/Searle, Des Plains, IL. Unlabeled glutamine and other amino acids were purchased from Sigma Chemical, St. Louis, MO. Cellulose filters (0.65-μm pore size) were obtained from Sartorius Filters, Inc., Hayward, CA. Sprague-Dawley adult male and littermate rats were purchased from Sasco Laboratories, Omaha, NE.

Preparation for Basolateral Membrane Vesicles. Adult, weanling (3 weeks old), and suckling rats (2 weeks old) were killed by cervical spine dislocation. The jejunal basolateral membrane vesicles (BLMV) were prepared using a modified centrifugation technique and separation on a Percoll gradient (9). The entire jejunum was removed and flushed with ice-cold normal saline and then filled with warmed (37°C) Buffer II solution which contained 1.5 mM KCl, 96 mM NaCl, 8 mM KH_2PO_4, 5 mM Na_2HPO_4, 27 mM Na_3 citrate, and 2 mM dithiothreitol (pH 7.4). The segments were then clamped and incubated for 15 min in a shaking water bath at 37°C. The segments were then emptied and

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[1] To whom requests for reprints should be addressed at MCN-D4116, Vanderbilt Medical School, Nashville, TN 37232.

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filled with ice-cold Buffer IV which contained 250 m*M* mannitol and 12 m*M* Hepes-Tris buffer (pH 7.4) and were gently palpated over ice for 3 to 5 min. The contents were then drained into a beaker and the volume brought up to 150 ml with Buffer IV and followed by centrifugation at 272*g* for 15 min. The cell pellet was then homogenized in 150 ml of Buffer IV in an Omni mixer for 3 min followed by centrifugation at 2450*g* for 20 min. The pellet was then discarded and the supernatant centrifuged at 22,000*g* for 25 min. The resulting fluffy layer of the pellet was resuspended in Buffer IV and homogenized (20 strokes) in a glass Teflon homogenizer. Ninety-five grams of the resulting homogenate was then mixed with 13.5 g of Percoll (Pharmacia) and centrifuged at 48,400*g* without brakes for 75 min. The distinct band of basolateral membrane was then aspirated with a transfer pipette and diluted 1/4 with Buffer V containing 100 m*M* mannitol, 100 m*M* KCl, and 12 m*M* Hepes-Tris buffer (pH 7.4). The homogenate was then centrifuged at 48,400*g* for 20 min. The resulting pellet was resuspended in the transport Buffer III (contained 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer, pH 7.4) and centrifuged at 20,000*g* for another 20 min followed by resuspension of the resulting pellet with a 25-gauge needle in the desired amount of the transport medium (Buffer III).

Purity of membrane vesicles preparation was assessed by the measurement of Na^+-K^+-ATPase according to the method of Scharschmidt *et al.* (10) and cytochrome *c* oxidase and NADPH-cytochrome *c* reductase as described by Beaufay *et al.* (11) and disaccharidases as described by Dahlquist (12). Protein assay was done according to the method of Lowry *et al.* (13) using bovine serum albumin as a standard.

Transport Measurement. Uptake by [^{3}H]glutamine by basolateral membrane vesicles was measured by a rapid filtration technique (14). All experiments were performed at 25°C unless specified otherwise in the figure legends. Transport was initiated by the addition of 20-μl aliquots of membrane vesicles suspension to 80 μl of the desired incubation medium containing [^{3}H]glutamine as described in the figure legends. At the desired time intervals, the reaction was stopped by the addition of 1 ml of ice-cold stop solution containing 100 m*M* mannitol, 100 m*M* NaCl, and 20 m*M* Hepes-Tris (pH 7.4). The suspension containing the vesicles was immediately pipetted into the middle of a cellulose nitrate filter (0.65-μm pore size, Sartorius filters) kept under suction and immediately washed with 5 ml of ice-cold stop solution. The filters were then dissolved in 5 ml of ready protein scintillation fluid (Beckman) and the remaining radioactive substrate was determined in a scintillation counter (Beckman Instruments, Palo Alto, CA). Radioactivity remaining in the filters, after pipetting an identical solution without vesicles, was used as background and subtracted from the uptake data. The uptake data were expressed in nanomoles per milligram protein per unit time.

Statistical Evaluation. All experiments were performed in triplicate and repeated two to three times. All data were analyzed statistically and expressed as mean ± SE. The two-tailed, unpaired Student's *t* test was used to evaluate the significance of data differences where multiple comparisons were made. The analysis of variance test was also used. A $P < 0.05$ was considered statistically significant.

Results

Purity of Membrane Vesicles. The basolateral membrane enzyme marker Na^+-K^+-ATPase showed a 9- to 10-fold enrichment in basolateral membrane vesicles suspension of all age groups compared with mucosal homogenate. There was an impoverishment in brush border membrane vesicles marker (leucine aminopeptidase and disaccharidase), mitochondrial marker (cytochrome *c* oxidase), and endoplasmic reticulum marker (NADH-cytochrome *c* reductase) (15).

Glutamine Transport versus Binding. To determine whether glutamine uptake represents binding or transport into the intravesicular space, two experiments were carried out in 3-week-old rats. An osmolality study was done in which the intravesicular space was decreased by increasing the osmolarity of the incubation medium from 200 to 700 mOsm. The uptake of glutamine was then determined under equilibrium condition (10 min). As shown in Figure 1, glutamine uptake was inversely related to Medium 1/osmolarity, according to the equation $Y = 0.4 \times +0.07$ with correlation coefficient of 0.92. In a temperature study, as shown in

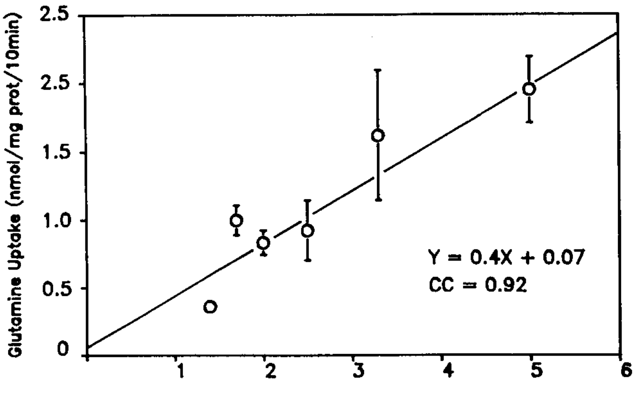

Figure 1. Effect of media osmolality on glutamine uptake. BLMV from weanling rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). The reaction was started by the addition of 20 μl of vesicles to a variable incubation medium containing 100 m*M* NaCl and varying concentrations of mannitol (0–500) to yield osmolarity from 200 to 700 in addition to 0.25 μCi of [^{3}H]glutamine and 2 m*M* glutamine (final concentration of glutamine was 0.02 m*M*). The reaction was stopped at 10 min. Experiments were done in triplicate and the data represent the mean ± SE.

Figure 2, glutamine uptake was significantly greater at 25°C compared with 0°C ($P < 0.05$–0.001 for all values).

Effect of Na$^+$ and K$^+$ Gradients on Glutamine Uptake. Glutamine uptake was determined in adult, suckling, and weanling rats under inwardly directed sodium and potassium gradients. Figure 3 depicts glutamine uptake in suckling and weanling rats. As seen, the uptake of glutamine was stimulated by both Na$^+$ and K$^+$ gradient with an "overshoot" phenomenon which occurred with Na$^+$ gradient only. In addition, the peak of overshoot was greater in suckling rats compared with weanling and adult rats, 0.71 ± 0.004 vs 0.50 ± 0.03 vs 0.23 ± 0.02 nmol/mg protein, respectively ($P < 0.001$).

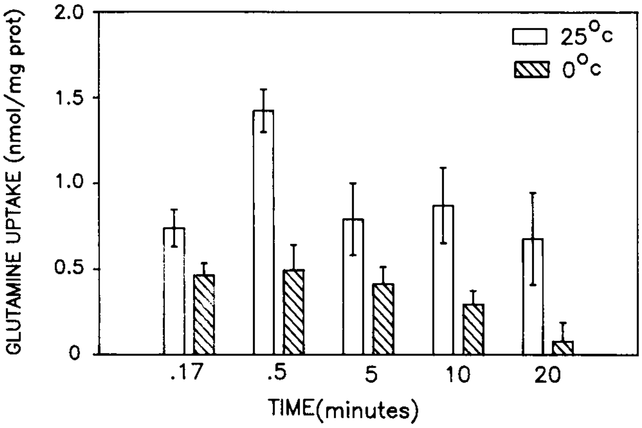

Figure 2. Effect of temperature on glutamine uptake. BLMV from weanling rats were prepared in 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Reaction was started by the addition of 20 μl of membrane vesicles to medium containing 100 m*M* NaCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris buffer (pH 7.4), 2 m*M* glutamine, and 0.25 μCi of [^{3}H]glutamine (final concentration of glutamine was 0.02 m*M*). Reaction was stopped at the time interval shown in the figure. Experiments were done in triplicate and the data represent the mean ± SE.

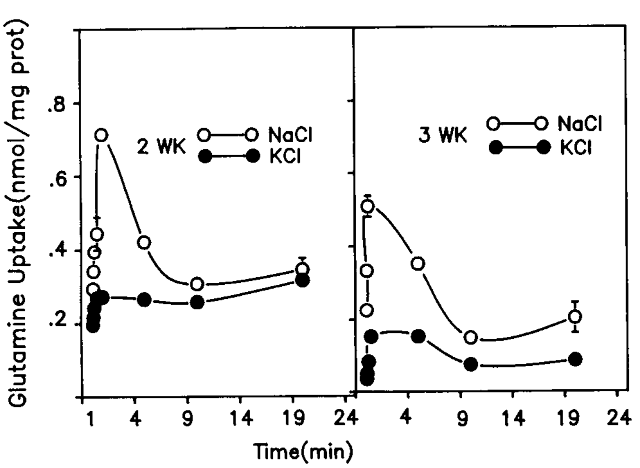

Figure 3. Effect of Na$^+$ and K$^+$ gradient on glutamine uptake. BLMV from suckling and weanling rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). The reaction was started by the addition of 20 μl of vesicles to a medium containing either 100 m*M* NaCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris (pH 7.4) or 100 m*M* KCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris (pH 7.4) in addition to 2 m*M* glutamine and 0.25 μCi of [^{3}H]glutamine (final concentration of glutamine was 0.02 m*M*). Reaction was stopped at the time interval shown in the figure. Experiments were done in triplicate and the data represent mean ± SE.

Effect of pH on Glutamine Uptake. In this study, glutamine uptake was determined in weanling rats under variable incubation medium pH that ranged from 5.5 to 9.0. As shown in Figure 4, maximal uptake occurred at a pH of 7.0, which marked inhibition at 5.5 and 9.

Kinetics of Glutamine Uptake. Glutamine uptake was determined in suckling, weanling, and adult rats under initial rate conditions. The substrate concentration ranged from 0.1 to 0.7 m*M* in the presence of inwardly directed Na$^+$ and K$^+$ gradients. These concentrations are with the normal physiologic concentration of glutamine in the blood of rats. A saturable process was observed under both Na$^+$ and K$^+$ gradient in the three age groups. As shown in Figure 5, under Na$^+$ gradient, mean V_{max} values in suckling rats was significantly greater compared with corresponding mean values in weanling and adult rats, 0.86 ± 0.17 vs 0.64 ± 0.08 and 0.41 ± 0.09 nmol·mg protein^{-1}·10 sec^{-1}, respectively ($P < 0.001$), while the K_m was similar at 0.3 ± 0.14 vs 0.3 ± 0.09 m*M* and 0.41 ± 0.09 m*M*, respectively.

As depicted in Figure 6, under K$^+$ gradient, a saturable process occurred. The V_{max} is similarly higher in suckling compared with weanling and adult rats, 0.68 ± 0.08 vs 0.50 ± 0.03 and 0.24 ± 0.03 nmol·mg protein^{-1}·10 sec^{-1}, respectively ($P < 0.001$), while the K_m was similar at 0.3 ± 0.08 vs 0.4 ± 0.05 and 0.27 ± 0.08 m*M*, respectively.

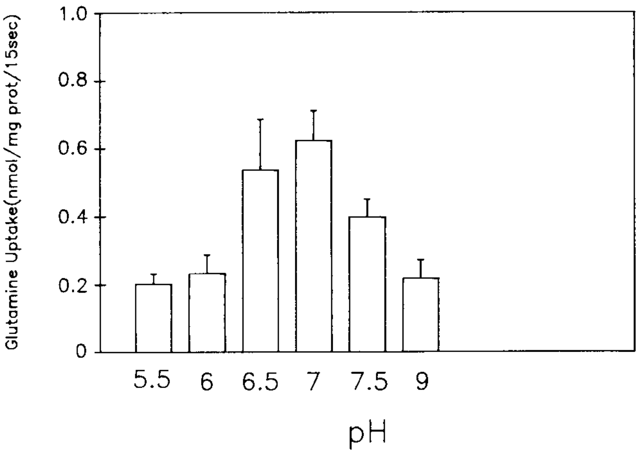

Figure 4. Effect of pH on glutamine uptake. BLMV from weanling rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). The incubation medium consisted of 100 m*M* NaCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris buffer (pH ranged from 5.5 to 9) in addition to 2 m*M* glutamine and 0.25 μCi of [^{3}H]glutamine (final concentration of glutamine was 0.02 m*M*). Reaction was stopped by the addition of 20 μl of membrane vesicles and stopped at 30 sec. Experiments were done in triplicate and the data represent the mean ± SE.

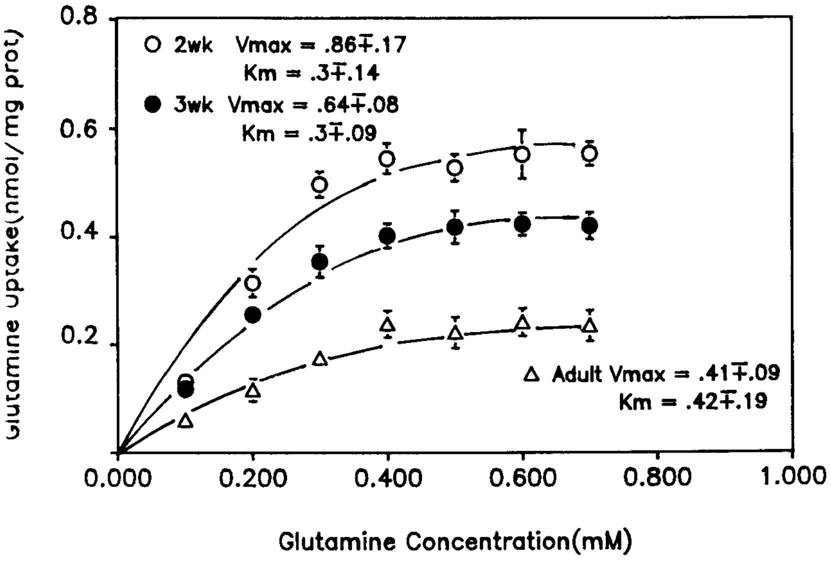

Figure 5. Kinetics of the Na^+-dependent system. BLMV from suckling, weanling, and adult rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Reaction was started by the addition of 20 μl of BLMV to incubation medium containing 100 m*M* mannitol, 100 m*M* NaCl, 20 m*M* Hepes-Tris buffer (pH 7.4), and 0.25 μCi of [^{3}H]glutamine in addition to variable glutamine concentrations ranging from 0.1 to 0.7 m*M*. The reaction was stopped at 15 sec. Experiments were done in triplicate and the data represent the mean ± SE.

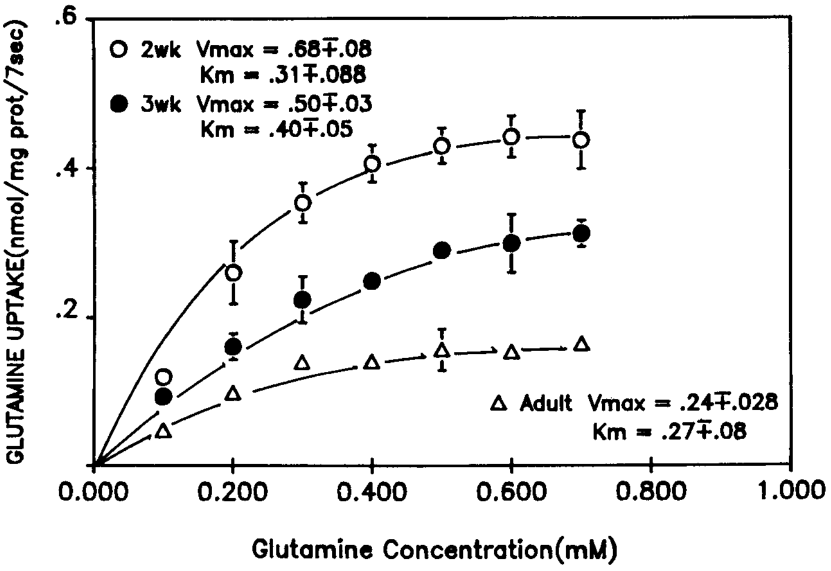

Figure 6. Kinetics of the Na^+-independent system. BLMV from suckling, weanling, and adult rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris buffer (pH 7.4). Reaction was started by the addition of 20 μl of BLMV to incubation medium containing 100 m*M* mannitol, 100 m*M* KCl, 20 m*M* Hepes-Tris buffer (pH 7.4), and 0.25 μCi of [^{3}H]glutamine in addition to variable glutamine concentrations ranging from 0.1 to 0.7 m*M*. The reaction was stopped at 15 sec. Experiments were done in triplicate and the data represent the mean ± SE.

Effect of Membrane Potential on Glutamine Uptake. To determine the effects of electrical potential on glutamine uptake, two studies were conducted in weanling rats. In the first study, valinomycin, a K^+ selective ionophore that mediates the electrogenic movement of K^+ against its concentration gradient, was used to maintain a negative membrane potential.

As shown in Figure 7, the uptake of glutamine was significantly higher with negative interior membrane potential (K^+ inside > K^+ outside + valinomycin) compared with the voltage-clamped condition (K^+ inside = K^+ outside + valinomycin) ($P < 0.005$ for all values).

In the second study, the uptake of glutamine was determined under anions with different lipid permeabilities such as sodium thiocyanate, sodium chloride, and sodium sulfate. As shown in Figure 8, under sodium thiocyanate gradient, a highly permeable anion, glutamine uptake is higher than under the less permeable anions, sodium chloride and sodium sulfate. A similar study conducted under inwardly directed K^+

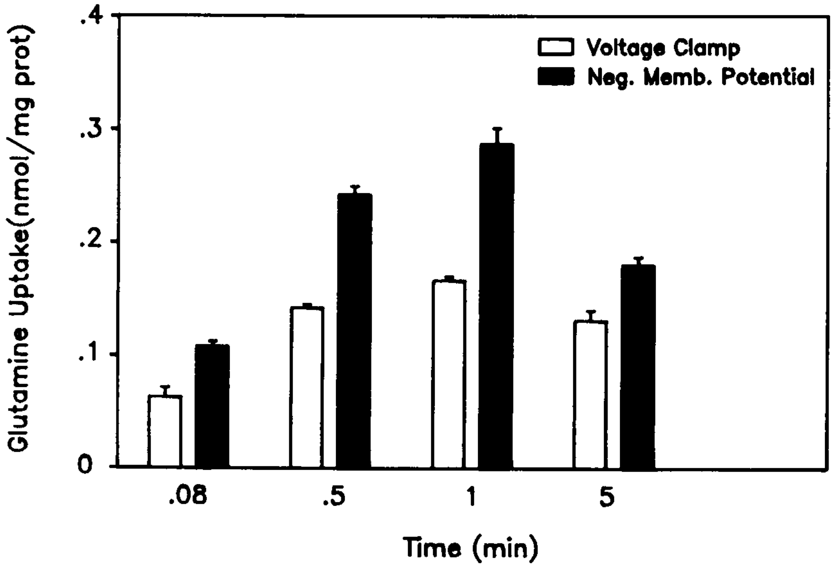

Figure 7. Effect of membrane potential on glutamine uptake. BLMV from weanling rats were preincubated in 50 m*M* KCl, 200 m*M* mannitol, and 20 m*M* Hepes-Tris buffer (pH 7.4). The incubation medium contained either 100 m*M* NaCl, 50 m*M* KCl, 20 m*M* Hepes-Tris (pH 7.4), 0.25 μCi of [^{3}H]glutamine, 2 m*M* glutamine (final concentration of glutamine was 0.02 m*M*), and valinomycin 10 μg/mg protein (voltage clamp) or in a medium similar to that described above, except that the KCl is reduced to 10 m*M* (negative membrane potential). Reaction was started by the addition of 20 μl of BLMV and stopped at the time intervals shown in the figure. Experiments were done in triplicate and the data represent the mean ± SE.

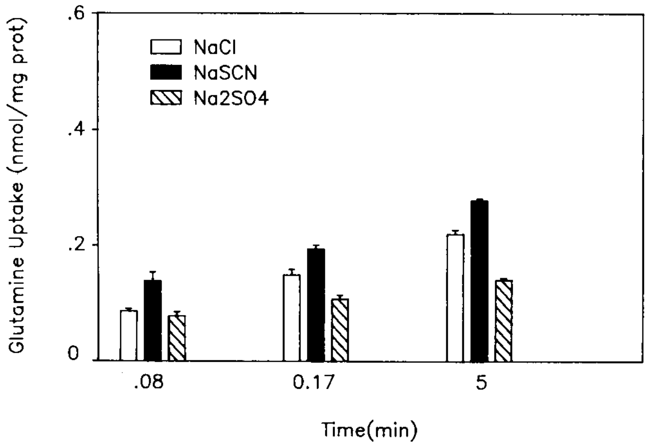

Figure 8. Effect of anion substitution on glutamine uptake. BLMV from weanling rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). Reaction was started by the addition of 20 μl of BLMV to incubation medium containing 100 m*M* of either NaCl, NaSCN, or $NaSO_4$ in addition to 100 m*M* mannitol, 20 m*M* Hepes-Tris (pH 7.4), 2 m*M* glutamine, and 0.25 μCi of [^{3}H]glutamine (final concentration of glutamine was 0.02 m*M*). Reaction was stopped at different time points. Experiments were done in triplicate and the data represent the mean ± SE.

gradient showed that anion substitution did not influence the rate of glutamine uptake.

These studies indicate that glutamine uptake under Na^+ gradient is electrogenic in nature. Similar experiments with potassium thiocyanate and potassium chloride showed no significant differences in glutamine uptake, 0.12 ± 0.01 and 0.11 ± 0.01 nmol·1 mg protein^{-1}·10 sec^{-1}, indicating an electroneutral process.

Effect of Other Amino Acids on Glutamine Uptake. As shown in Table I, under Na^+ gradient, glutamine uptake was inhibited by several amino acids. Inhibition by α-methylaminoisobutyric acid is indicative of System A. However, it is also exhibited tolerance to System N substrates (histidine and asparagine) and System ACS substrates (cysteine and serine).

Under K^+ gradient, as depicted in Table II, several amino acids exhibited minimal inhibition; however, leucine and 2-aminobicyclo(2,2,2)hepatane-2 carboxylic acid exhibited greater inhibition. This pattern is indicative of an L-like system.

Effect of Lithium Substitution on Glutamine Uptake. In this study, glutamine uptake was determined under inwardly directed Na^+, lithium, and choline gradients. As depicted in Figure 9, the uptake of glutamine at the basolateral membrane exhibited tolerance for lithium and not for choline.

Discussion

The uptake of glutamine by the basolateral membrane vesicles of suckling, weanling, and adult rats was studied by a well-established rapid filtration technique. Previously, we have published a full characterization of glutamine uptake by basolateral membranes of the adult rat intestine (17). The purpose of this article was to define the developmental changes in the transport of glutamine by suckling and weanling rats and compare the data with values obtained for adult rats. The experiments reported here, which include adult rat preparation, were done along with preparations of BLMV from suckling and weanling rats.

The osmotic sensitivity study, as shown in Figure

Table I. Percentage of Inhibition of [^{3}H]Gln Uptake by Various Amino Acids with Inwardly Directed Na$^+$ Gradient[a]

Rats	Control (%)	Gln (%)	Gly (%)	Met (%)	Phe (%)	MeAIB (%)	Ala (%)	Asn (%)	His (%)	Leu (%)	BCH (%)	Ser (%)	Cys (%)
Adult	100	66	5	8	10	70	38	87	52	20	0	71	78
3-week old	100	59	30	24	7	62	18	47	60	24	9	36	26
2-week old	100	72	50	18	10	70	18	43	50	28	26	36	49

[a] BLMV from suckling, weanling, and adult rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). The incubation medium consisted of 100 m*M* NaCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris (pH 7.4). In addition to 0.02 m*M* glutamine, 0.25 μCi of [^{3}H]glutamine and various amino acids were added at a concentration of 20 m*M*. The reaction was started by the addition of 20 μl of vesicles to the incubation medium and the reaction was stopped at 15 sec. Experiments were done in triplicate and the data represent the mean ± SE.

Table II. Percentage of Inhibition of [^{3}H]Gln Uptake by Various Amino Acids with Inwardly Directed K$^+$ Gradient[a]

Rats	Control (%)	Gln (%)	Gly (%)	Met (%)	Phe (%)	MeAIB (%)	Ala (%)	Asn (%)	His (%)	Leu (%)	BCH (%)	Ser (%)	Cys (%)
Adult	100	67	0	18	25	0	25	26	7	42	52	28	0
3-week old	100	56	15	19	22	0	11	1	0	46	56	14	2
2-week old	100	79	30	0	30	46	25	39	32	70	60	17	12

[a] Brush border membrane vesicles from suckling, weanling, and adult rats were preincubated in 300 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). The incubation medium consisted of 100 m*M* KCl, 100 m*M* mannitol, and 20 m*M* Hepes-Tris (pH 7.4). In addition to 0.02 m*M* glutamine, 0.25 μCi of [^{3}H]glutamine and various amino acids were added at a concentration of 20 m*M*. The reaction was started by the addition of 20 μl of vesicles to the incubation medium and the reaction was stopped at 15 sec. Experiments were done in triplicate and the data represent the mean ± SE.

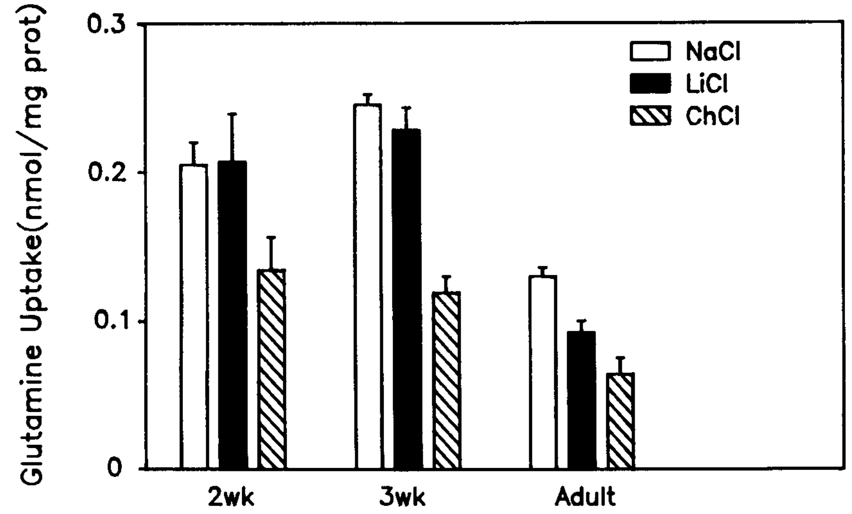

Figure 9. Effect of lithium substitution on glutamine uptake. BLMV from suckling, weanling, and adult rats were prepared in 300 m*M* mannitol and 20 m*M* Hepes-Tris (pH 7.4). Reaction was started by the addition of 20 μl of BLMV to incubation medium containing 100 m*M* of either NaCl, LiCl, or choline chloride in addition to 100 m*M* mannitol, 20 m*M* Hepes-Tris buffer (pH 7.4), 2 m*M* glutamine, and 0.25 μCi of [^{3}H] glutamine (final concentration of glutamine was 0.02 m*M*). Reaction was stopped at 1 min. The experiments were done in triplicate and the data represent the mean ± SE.

1, indicated that the uptake of glutamine represents mainly a transport into the intravesicular space rather than mere binding to the membrane surface. The active nature of this process is verified by a temperature-dependency study that showed a significant inhibition at 0°C compared with 25°C (16).

The uptake of glutamine was stimulated under inwardly directed Na$^+$ gradient with overshoot phenomenon. The magnitude of the overshoot was significantly greater ($P < 0.001$) in suckling compared with corresponding mean values in weanling and adult rats. This higher rate of uptake in suckling compared with weanling and adult rats is secondary to increase number and/or activity of transporters as shown by higher V_{max}. The greater V_{max} may represent a higher demand in period of rapid cellular proliferation and differentiation and correlates with the findings of Pinkus and Windmueller (8) of a higher glutaminase specific activity in suckling rats and with the finding of Kimura (7) of a higher glutamine oxidation of CO_2 by jejunal slices of suckling compared with adult rats.

Kinetic studies showed a saturable process under both Na^+ and K^+ gradient, indicating the existence of a Na^+-dependent and Na^+-independent transport system in the three age groups. However, the V_{max} for both systems was higher in suckling compared with weanling and adult rats. The Na^+-dependent system is electrogenic and pH dependent whereas the Na^+-independent system is electroneutral. Similar findings of two transport systems with higher activity in the suckling rats was found for brush border membrane preparation (18). However, the V_{max} and K_{max} for the sodium-dependent and independent processes in both suckling and weanling rats were significantly greater in brush border membranes compared with the basolateral membranes.

These systems were characterized further with inhibition studies. As shown in Table I, under Na^+ gradient, glutamine uptake was inhibited by several amino acids. The significant inhibition by MeAIB indicates, similar to other plasma membranes, a transport by System A. However, the inhibition by asparagine, histidine, serine, and cysteine indicated tolerance to System N and System ACS as well. The tolerance to System N substrates is supported by the lithium substitution study which is seen with System N.

The inhibition study under K^+ gradient similarly showed inhibition of glutamine uptake by several amino acids but more so with leucine and BCH, suggesting the presence of an L-like system.

We conclude that glutamine uptake in the basolateral membrane undergoes developmental changes consistent with more activity in suckling compared with and adult rats. In all age groups, glutamine is transported by two transport systems. The Na^+-dependent A system with tolerance to System N and System ASC substrates. The Na^+-independent system transported leucine and BCH and correlated with the classical L system.

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