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L-Alanine transport in small intestine brush-border membrane vesicles of obese rats

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Abstract

Membrane vesicles from the small intestine brush border were obtained and used to determine the possible effects of genetic or nutritional obesity on L-alanine uptake. Membrane vesicles from Zucker *fa/fa* obese rats and cafeteria diet-fed Zucker Fa/? rats showed the same characteristics as those of standard diet-fed lean animals. All preparations showed sodium-dependent transport as the main pathway for L-alanine uptake within the substrate concentration range tested. The apparent substrate affinity constant (K_m) values and the pattern of inhibition of Na^+-dependent L-alanine uptake by other amino acids (L-leucine and L-glutamine), suggests that system B involved in the transport of dipolar amino acids (formerly named Neutral Brush Border System) participates in the Na^+-dependent transport of L-alanine. The affinity constant (K_m) for L-alanine was essentially the same for all the groups studied (in the range of 10 mM). However, there was a higher ($P < 0.05$) maximal capacity (V_{max}) in preparations from diet-induced obese animals (cafeteria diet) than that of genetically obese rats. These results indicate that either nutritional or genetic obesity may modify the capacity but not the affinity of transport systems for L-alanine uptake in the brush border of rat small intestine.

Key words: Brush-border membrane; Amino acid transport; Obesity; (Rat)

1. Introduction

Obesity is characterized by an inordinate accumulation of lipids in white adipose tissue [1], whatever the diverse causes that lead to this accumulation [2]. Rodents are frequently used to study the metabolic and nutritional consequences of obesity, either induced by dietary manipulation or with a genetic basis. One of the most common models of hypercaloric diet-induced obesity is based on the *cafeteria diet*, in which the rats select their food from a number of different palatable items; thus, increasing voluntarily their caloric ingestion without any additional manipulation or force-feeding [3]. Cafeteria diets are hyperlipidic and induce an accumulation of fat [4], with enhanced heat production by brown adipose tissue [5]. Cafeteria diets are known to increase N retention [6], probably as a consequence of an increased ability to absorb dietary amino acids [7], complemented by lower urea cycle activity [8] and, as a consequence, decreased nitrogen elimination in the urine [9].

Genetically obese (Zucker *fa/fa*) rats accumulate large amounts of adipose tissue [10]; since *fa/fa* rats are hyperphagic, they ingest higher amounts of N than lean controls [6], although the nitrogen retained is similar for both groups because of the higher excretion rates of *fa/fa* rats [7]. Genetically obese rats absorb the same percentage of amino acids from the diet, as do the lean animals, and thus sustain high protein accretion rates for longer periods during development [7], than lean control or the cafeteria-fed rats.

Amino acid transport in rat small intestine has been studied and characterized both in brush-border [11,12] and or in baso-lateral membranes [13] from animals subjected to different nutritional [14] or developmental [15] situations. Although there is much of information available on intestinal amino acid transport, there are no references on the possible effects of genetic or hypercaloric diet-induced obesity on amino acid transport. The aim of this study is the kinetic characteriza-

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tion of amino acid uptake in the isolated brush-border membrane vesicles of obese rats, centered on L-alanine uptake, since this is one of the amino acids that are taken up most efficiently by rats fed hypercaloric diets [7].

2. Methods

2.1. Animals

Zucker lean and obese rats (from Charles River stock, USA) were used. They were bred at the Animal Service of the University of Barcelona under controlled conditions of light (12 h on/12 h off), humidity (70–80%) and temperature (20–21°C). The rats were housed in polypropylene-bottomed cages with wood shavings as absorbent material. After delivery, lactating dams and their litters were randomly divided in two groups. One group was fed ad libitum with standard laboratory chow diet (Panlab A03/A04, Barcelona, Spain) and tap water. The second group was fed a fat-rich hypercaloric diet (*cafeteria diet*) with daily fresh offering of biscuits spread with liver pâté, bacon, chow pellets, tap water and whole milk enriched with 300 g/l of sucrose plus 10 g/l of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica, Spain). All materials were previously weighed and presented in excess (approx. 20–30% over the expected consumption). This diet is a simplified version of an earlier diet developed and studied by us [16]. Rats were weaned on day 21, and the males were picked up and fed with the described diets, up to day 60. Zucker obese (*fa/fa*) rats were obtained from standard diet-fed litters, were weaned on day 21 and fed the standard diet up to day 60.

The composition of the diets was the following: *Cafeteria diet* fed rats derived about 30% of dietary energy from fats, 56% from carbohydrate and 14% from protein. Rats fed with standard diet, obtained 9% of energy from fats, 69% from carbohydrate and 22% from protein.

2.2. Isolation of brush-border membrane vesicles

Brush-border membrane vesicles were prepared by selective precipitation induced by divalent cations [17]. Rats were killed by decapitation at the beginning of the light cycle, and the small intestine was dissected and rinsed with ice-cold 9 g/l KCl. The intestine was weighed and opened longitudinally in order to scrape off the mucosa, which was resuspended in 10 volumes of cold 2 mM Tris-(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) (pH 7.5) solution containing 300 mM mannitol and 10 mM phenylmethanesulphonyl fluoride (PMSF), and homogenized for 15 s with a Polytron (Kinematica, Switzerland); $MgCl_2$ was added to a final concentration of 10 mM and the solution was then stirred for 5 min and left to stand for 15 min. The suspension was then centrifuged (15 min, $3000 \times g$); the pellet was discarded, and the supernatant was centrifuged again (30 min, $30000 \times g$). The final pellet was resuspended using a syringe (35G gauge needle) in an ice-cold buffer solution 10 mM *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulphonic acid) (Hepes)-KOH (pH 7.5) containing 300 mM mannitol. The last two steps were repeated once. The pellet, which contained the purified brush-border membrane vesicles, was finally resuspended in the same buffer so as to obtain a protein concentration of 4–5 mg/ml; this suspension was used directly in the transport assays. All procedures were carried out at 4°C.

2.3. Uptake measurements

Alanine transport assays were performed at 27°C following the procedure described by Hopfer et al. [18]. Aliquots of 10 μl of isolated vesicles suspended in buffer were mixed with 40 μl of buffered – 10 mM Hepes-KOH (pH 7.5) – solution containing 100 mM sodium or potassium thiocyanate, and L-[^{3}H]alanine ranging from 0.05 to 20 mM. Mannitol was added to maintain isosmotic conditions. In the competitive assays, different L-amino acids were added to a final concentration of 50 mM. At the end of the incubation time (5 s, except for the time-course assays) alanine transport was stopped by the addition of 1 ml of ice cold stop solution containing 100 mM mannitol, 100 mM NaCl and 10 mM Hepes-KOH (pH 7.5). The full volume was filtered across nitrocellulose filters (0.45 μm mean pore) (Schleicher & Schuell, Germany) under vacuum and washed with 5 ml of stop buffer. The filters were set in mini vials and their radioactivity was determined using a scintillation counter. The non-specific retention of labelled alanine in the filters was determined using blanks, assayed in the absence of vesicles, and then subtracted of the total retention data. Transport assays were carried out in triplicate using vesicles from 4–6 different rats.

The possible effects of ions on L-alanine uptake, were studied using incubating buffer where sodium and potassium thiocyanate were substituted by an equimolecular amount of choline or lithium or sodium or potassium chloride.

Protein content was determined in homogenates and membrane preparations with the method of Lowry et al. [19]. Alkaline phosphatase (EC 3.1.3.1) [20] and sucrase (EC 3.2.1.48) [21] activities were determined as brush-border membrane markers. Na^+/K^+-ATPase (EC 3.6.1.3) [22] was measured as a baso-lateral membrane marker and glucose-6-phosphatase (EC 3.1.3.9) [23] was used as an endoplasmic reticulum marker.

2.4. Chemicals

Labelled L-[^{3}H]alanine was purchased from NEN-Research Products (DuPont, France). Other reagents (high quality grade) were obtained from Sigma (USA) and Merck (Germany).

2.5. Kinetic analysis and statistics

Kinetic analysis (linear regression) was performed with the Fig P program (Fig P Corporation, USA). Statistical comparisons were carried out with Student's *t*-test.

3. Results

On day 60, the body weights of rats were: 217 ± 7.1 g, 283 ± 5.2 g and 332 ± 7.6 (mean ± S.E.; $n = 10$), respectively for control, cafeteria-fed and genetically obese groups. All groups were different ($P < 0.001$) with respect to that parameter. The different growth pattern experienced by the three groups also implied the existence of significant differences ($P < 0.001$) in the weight of the small intestine: 7.93 ± 0.21 g, 9.9 ± 0.33 g and 13.1 ± 0.2 g, respectively, although the intestinal weight/body weight ratios were similar: about 3.6%.

The percentage of recovery and relative specific activities (enrichment) of the marker enzymes are shown in Table 1. The enrichment values of brush-border membrane marker enzymes – alkaline phosphatase and sucrose – were higher than those of non apical membranes – Na^+/K^+-ATPase and glucose-6-phosphatase –. Thus, the brush-border membrane preparations from three groups showed similar levels of contamination by other cell membranes.

Fig. 1 shows the time-course pattern of L-alanine uptake by isolated brush-border membrane vesicles. The uptake in a sodium-rich medium was 3–4-fold higher than that found in a potassium-rich medium at short incubation times: cafeteria-fed group showed higher uptake rates than control or *fa/fa* rats. Maximal alanine uptake rates were found – for all groups – for 5 and 10 s incubations; total transport, measured in the presence of sodium, showed a near-linear pattern during this period. For that reason routine transport assays were carried out with 5s incubation. Alanine uptake measured in a sodium-free medium showed closely similar patterns for all cases, indistinguishable from those found with sodium containing-medium at equilibrium.

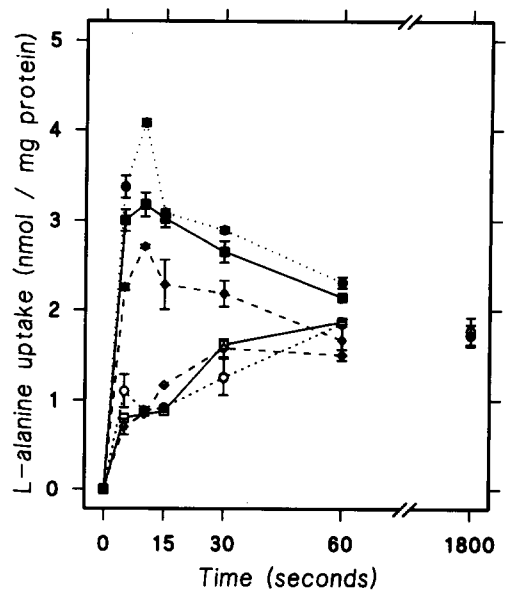

Fig. 1. Time-course of L-alanine uptake by rat small intestine brush-border membrane vesicles. Membrane vesicles were prepared in buffer containing 300 mM mannitol and 10 mM Hepes (pH 7.5) and were incubated at 27°C in a medium of the same pH containing 5 mM L-[3-^{3}H]alanine (91 MBq/mmol), 100 mM mannitol and 100 mM KSCN (□, control; ○, cafeteria; ◇, genetic obesity) or NaSCN (■, control; ●, cafeteria; ◆, genetic obesity), in final concentrations. Data were expressed as the means ± S.E. of valorations made in triplicate from three animals of each group.

The transport ratio of L-alanine was maximal in the presence of sodium salts, especially in the presence of thiocyanate, as can be seen in Fig. 2. There is no evidence to support any activation of alanine uptake by potassium or lithium salts in brush-border membrane vesicles from rat intestine.

Table 1
Enrichment and recovery values of marker enzyme activities

	Group	Sucrase	Alkaline P_{ase}	Na^+/K^+-ATP_{ase}	Glucose-6-P_{ase}
Enrichment	control	15.3 ± 1.2	8.3 ± 1.2	0.5 ± 0.05	2.4 ± 0.3
	cafeteria	12.5 ± 1.6	7.5 ± 1.7	0.4 ± 0.04	2.1 ± 0.2
	genetic obesity	13.3 ± 1.2	8.0 ± 0.4	0.4 ± 0.05	2.3 ± 0.2
Recovery (%)	control	30.8 ± 5.3	16.7 ± 2.4	0.07 ± 0.01	4.9 ± 0.6
	cafeteria	22.2 ± 3.4	15.0 ± 3.4	0.06 ± 0.01	4.2 ± 0.4
	genetic obesity	26.9 ± 2.2	16.4 ± 0.8	0.06 ± 0.01	4.5 ± 0.4

Enrichment is the ratio of the marker enzyme activity present in the membrane preparation with respect to that found in the homogenate. Recovery is the percentage of activity found in the whole membrane preparation with respect to that of the initial mucose homogenate. Data are expressed as the means ± S.E. of valorations made in four animals of each group.

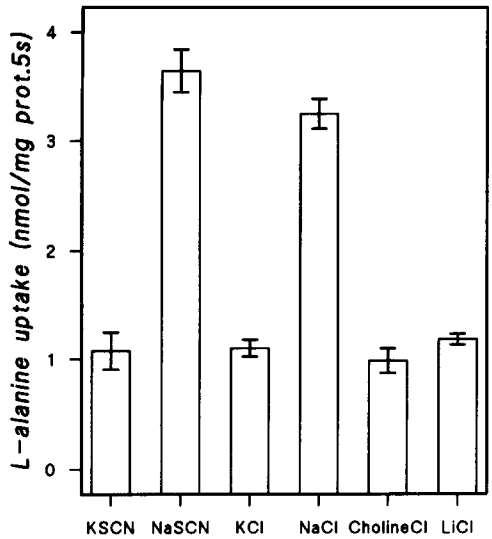

Fig. 2. Effect of different salts in the uptake of L-alanine by rat small intestine brush-border membrane vesicles of control group. Membrane vesicles were prepared in buffer containing 300 mM mannitol and 10 mM Hepes (pH 7.5) and were incubated at 27°C in a medium of the same pH containing 5 mM L-[3-^{3}H]alanine (91 MBq/mmol), 100 mM mannitol and 100 mM KSCN, NaSCN, KCl, NaCl, Choline-Cl or LiCl. Data were expressed as the means ± S.E. of valorations made in triplicate from three animals.

The kinetic pattern of L-alanine uptake by brush-border membrane vesicles is shown in Fig. 3. The uptake of alanine in the absence of sodium was almost linear with similar rates for all groups. On the other hand, total alanine uptake – measured in a sodium-rich medium – showed a non-linear non-saturable pattern; alanine uptake in the presence of sodium was higher than in its absence; i.e., at 10 mM alanine, the sodium-independent uptake was only about 25% of total alanine transport. The incorporation of alanine into vesicles from cafeteria-fed animals was higher than that of standard diet-fed rats; the differences being even more marked with reference to the genetically obese group.

Fig. 4a shows the Eadie-Hofstee plot of sodium-dependent L-alanine transport measured in brush-border vesicles from lean animals. The kinetic pattern of sodium-dependent transport gave a good linear corre-

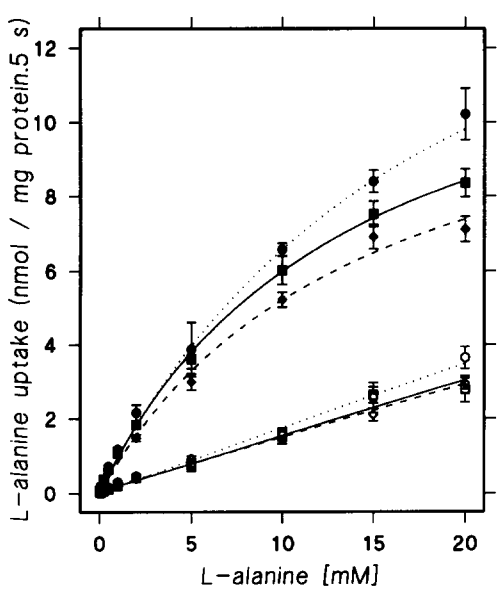

Fig. 3. Concentration dependence of L-alanine uptake. Membrane vesicles were prepared in 10 mM Hepes (pH 7.5) buffer containing 300 mM mannitol and were incubated for 5 s at 27°C in a medium of the same pH containing 0.05 mM to 20 mM L-[3-^{3}H]alanine (4.94 GBq to 33.3 MBq/mmol), KSCN (□, control; ○, cafeteria; ◇, genetic obesity) or NaSCN (■, control; ●, cafeteria; ◆, genetic obesity), in final concentration, and sufficient mannitol to obtain an osmolarity of 310 mosM. Data were expressed as the means ± S.E. of valorations made in triplicate from six animals in each group.

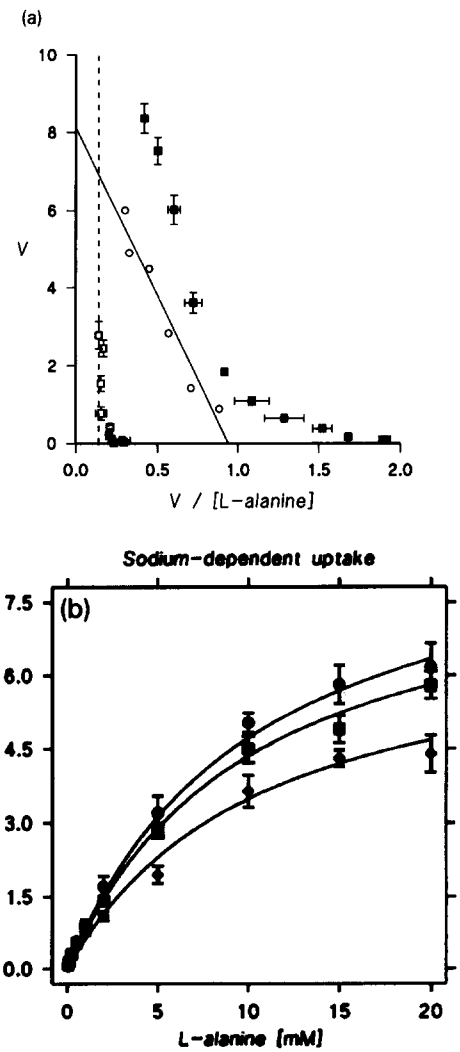

Fig. 4. (a) Eadie-Hofstee plot of L-alanine uptake by brush border vesicles of control group. The uptake was measured in a KSCN medium (□) (sodium-independent transport plus diffusion) or in a NaSCN medium (■) (sodium-dependent transport, sodium-independent transport plus diffusion). Deduced diffusional component (dashed line) and Na^+-dependent component (○). Units are: x, μl/mg protein per 5 s; y, nmol L-alanine/mg protein per 5 s. (b) Non-linear regression (Michaelis-Menten plot) of sodium-dependent uptake data from control (■), cafeteria (●) and genetic obesity (◆).

lation ($r = 0.91$), which increased when the values corresponding to low concentrations (lower than 1 mM) were not taken into account ($r = 0.98$). On the other hand, the alanine uptake measured in a sodium-free medium did not follow – at low concentrations – a linear pattern and showed saturable kinetics. This behaviour was also observed in vesicles from both types of obese rats. Fig. 4b shows, for better comparison, the Michaelis-Menten plot of the sodium-dependent uptake of the three groups.

Kinetic parameters of L-alanine uptake by isolated brush-border membrane vesicles are summarized in Table 2. Values of maximal uptake rate (V_{max}), affinity constant (K_m) and permeability coefficient (P.C.) were derived from linear regression of Eadie-Hofstee plots: sodium-dependent uptake was determined as the difference from alanine uptake measured in the Na^+-free medium with respect to that in the Na^+-rich medium; Na^+-independent uptake was estimated by subtracting the diffusion values from alanine uptake in Na^+-free medium, and diffusional component was derived from the linear regression of non-saturable values measured in the Na^+-free medium at high values of substrate concentration (as can be seen in Fig. 4). The affinity values of the transport components for L-alanine (K_m) are identical for all types of vesicles tested; however, the values of V_{max} showed significant differences ($P < 0.05$) for sodium-dependent component when comparing genetic and dietary obesity, the values for cafeteria-fed rats were higher than those for *fa/fa* rats. Sodium-independent alanine transport showed a higher affinity and lower capacity than the sodium-dependent transport: the values of K_m were 20-fold higher in the latter than in the former, and the values for V_{max} for sodium-independent were in the range of 1% of that of sodium-dependent transport.

Fig. 5 shows the inhibitory effect of different amino acids at saturable concentrations on the uptake of 0.05 mM L-alanine. The inhibition of sodium-dependent uptake was determined as follows:

$$I = 100 - \left(\left[V_i^{Na} - V_i^{K}\right]/\left[V^{Na} - V^{K}\right]\right) \cdot 100$$

where I represents the percentage of inhibition caused, V^{Na} and V^{K} represents the uptake rate in the presence or absence of sodium, respectively, V_i^{Na} and V_i^{K} represent the uptake rate in the presence of the inhibitor amino acid.

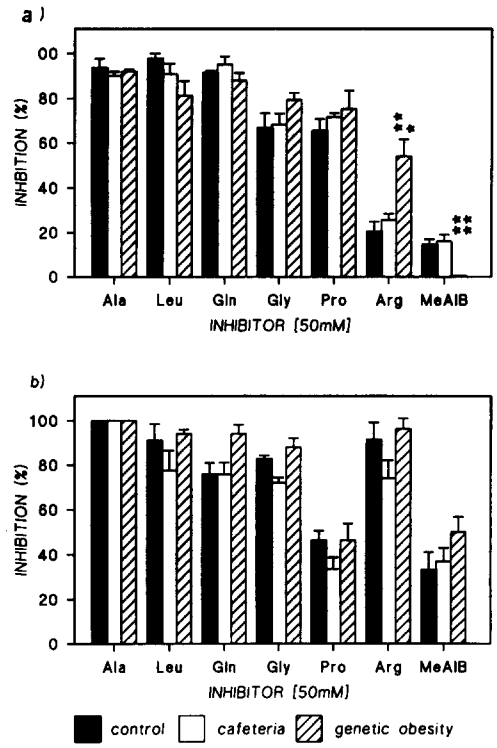

Fig. 5. Inhibition of L-alanine uptake by other L-amino acids. Brush-border vesicles were prepared in 10 mM Hepes (pH 7.5) buffer containing 300 mM mannitol and were incubated for 5 s at 27°C in a medium of the same pH containing, in final concentrations, 0.05 mM L-[3-^{3}H]alanine (12.3 GBq/mmol), 100 mM mannitol, 100 mM NaSCN or KSCN and 50 mM mannitol (basal uptake) or inhibitor. Data were expressed (as detailed in the text) as the mean of% inhibition caused by the different amino acids (means ± S.E.) from four or five animals. (a) Sodium-dependent uptake inhibition. (b) Sodium-independent uptake inhibition. Statistically significant differences between groups were found for L-Arg and MeAIB in the genetic obesity group: * $P < 0.05$; ** $P < 0.01$ versus control (solid stars) and cafeteria (open stars).

Alanine, leucine and glutamine produced the most marked effects on L-alanine transport, showing an inhibition higher than 85% of the basal transport. The percentage of inhibition caused by glycine and proline was lower (60–70%) but higher than that produced by

Table 2
Transport kinetic parameters of L-Alanine uptake by rat small intestine brush-border membrane vesicles

Group	P.C. (μl/mg protein per 5s)	V_{max} (nmol L-Ala/mg protein per 5s)		K_m (mM)	
		Na^+-independent	Na^+-dependent	Na^+-independent	Na^+-dependent
Control	0.14 ± 0.02	0.08 ± 0.03	8.7 ± 0.5	0.58 ± 0.15	10.1 ± 1.3
Cafeteria	0.16 ± 0.01	0.07 ± 0.02	9.6 ± 0.8	0.35 ± 0.09	10.4 ± 1.8
Genetic obesity	0.15 ± 0.01	0.09 ± 0.03	7.1 ± 0.7 *	0.41 ± 0.17	10.4 ± 2.7

Values are obtained as described in the text. Symbols are: P.C., permeability coefficient; V_{max}, maximal uptake capacity; K_m, apparent affinity constant for the substrate. * statistical significant difference ($P < 0.05$) vs. cafeteria group. Data are expressed as the means ± S.E. of six animals of each group.

arginine and methyl-aminoisobutyric acid (MeAIB), which was lower than 25%. This described pattern was followed by the vesicles from the three different groups, with the only exception of those from *fa/fa* rats for arginine and MeAIB; which presented statistically differences versus the control group.

The inhibition of sodium-independent uptake was determined as follows:

$$I = 100 - \left(\left[V_i^K - D\right]/\left[V^K - D\right]\right) \cdot 100$$

where I represents the percentage of inhibition caused, V^K is the uptake rate under the conditions of the assay, V_i^K is the uptake rate in the presence of the inhibitor amino acid, and D represents the diffusion rate.

The inhibition of sodium-independent transport followed the same pattern as the sodium-dependent transport, except for arginine, which inhibited alanine transport more strongly in the absence of sodium.

4. Discussion

The studies of dietary regulation of intestinal amino acid absorption has shown that either high [14,24] or low [25,26] protein diets increase the amount of amino acid absorbed. This pattern may be further complicated when individual differences between essential and non-essential amino acids are taken into account [27]. The different experimental approaches used in most studies do not clarify the relative importance of transport induction/repression by dietary protein or the permissive role of other nutrients. Thus, the fact that a hypercaloric, mainly hyperlipidic but highly palatable and slightly hypoproteic diet induces an increase in the absorption of different amino acids [7], suggests that diet may alter the function of amino acid transport systems. Attention should be paid to the *cafeteria* diet composition, since its protein proportion on a energy basis is about half that of standard diets [7]. However, the hyperphagia that cafeteria-fed rats develop [3] results typically in the ingestion of about the same amount of protein than control-fed rats [7]. In addition, if we consider that genetically obese rats absorb – in absolute terms– more dietary amino acids than cafeteria-fed rats [7], despite having lower absorptive efficiency, it is easy to infer that cafeteria-fed rats – or more properly, their small intestine – can adjust the different amino acid uptake pattern in response to diet composition; the possible modification of the activity of the amino acid transport systems may have a key role in this adaptive process.

The maintenance of the weight of small intestine relative to body weight of the cafeteria-fed rats when compared with standard diet-fed animals, suggests that their different nutritional treatment does not induce substantial modifications in the gross morphology of small intestine. The variations in the efficiency of absorption of amino acids [7] may be a consequence of changes in the absorption pattern and not due to alterations in the shape or mass of the intestine. The same argument seems to be applicable to genetically obese rats, since these animals are able to maintain the same body weight/intestinal weight ratio in the same range as lean rats.

Isolation of fully functional membrane vesicles from intestinal brush border was performed using conventional procedures [17,28] based on their selective separation in a magnesium-containing medium. The similar recovery values obtained for enzyme marker activities in all groups, the comparable enrichment values, the similar responses to hyperosmolar media and the similar values for intravesicular volumes (data not shown) concur in establishing the adequacy of the procedures used to obtain brush-border vesicles. The vesicle preparation allowed us to establish a fully comparable experimental setup for the measurement of amino acid transport. The values obtained for enrichment and recovery were in the same range as those described in the literature [29,30].

The pattern of alanine uptake by rat vesicles is very similar to that of rabbit vesicles [31]; in both cases, the maximal uptake ratio was found in a sodium-thiocyanate medium. This behaviour contrasts with the described presence of a potassium-dependent overshoot pattern for L-phenylalanine uptake in mouse intestine [32].

The higher response obtained in the time-course analysis of L-alanine uptake in a sodium-rich medium may be attributed to the presence of the B system (formerly named Neutral Brush Border) in the intestinal apical membrane. This sodium-dependent transport system has been widely described [33,34] and may be the main pathway for alanine uptake, since alanine is a typical zwitterionic amino acid. Furthermore, the coincidence in the values obtained for K_m and those described in membrane vesicles of rabbit [31], confirms the analogy of the B system illustrated here and that previously described [31]. The almost total inhibition in alanine uptake caused by glutamine and leucine – both specific substrates for the B system [33,35] in a sodium-rich medium – point towards the B system being the main way of entrance through the brush-border membrane. However, the relatively low inhibition of alanine uptake by glycine – another specific substrate for B system [33] – seems to indicate that alanine may be also taken up by other transport systems, secondary to the B system pathway. Thus, the possible interpretation of L-alanine uptake by brush-border membrane is further complicated by the inhibition patterns induced in alanine transport by some specific substrates of other transport systems, (a) the

high inhibitory effect of proline, a specific substrate for the IMINO system [36], (b) the capacity of arginine – a specific substrate for the y^+ system – to inhibit alanine uptake, especially in the Zucker *fa/fa* group; and finally, (c) the fact that MeAIB, a substrate of the IMINO system [33,36] partially inhibits the uptake of L-alanine in lean and dietary obese animals, but not in genetic obese animals. Thus, it can be argued that L-alanine may be taken up, partially, by other transport systems different from the B system: i.e., the IMINO and the y^+ systems may be involved in L-alanine uptake, in accordance with previous reports [37]. The inhibitory pattern induced by MeAIB, substrate of the A system, suggests the presence of this transport system in membrane vesicles of rat, in a similar way to that described for their presence in brush border from cat [38] and Guinea pig [39] intestine. However, right now there is not enough evidence to confirm or discriminate or reject this implication.

From Eadie-Hofstee representation of sodium-independent uptake may be deduced the presence of an additional transport system, other than diffusion, on L-alanine uptake, and confirms the postulated complexity of this component of transport [40]. Under these conditions, the marked inhibition by leucine – a specific substrate for L system under these conditions – and the partial inhibition caused by MeAIB, which is also transported by this system [34], suggests the additional participation of this system in the uptake of L-alanine, since the L system is an ubiquitous and broadly-tolerant system [36]. It may be interesting to take into account that the uptake of amino acids in small intestine seems to be shared by different systems, being the relative importance of each one determined by a number of different variables [33]. The possible role of sodium-independent transport may be related with nutrient absorption in the distal part of the small intestine, since its high affinity could facilitate the uptake of L-alanine, or perhaps other amino acids, when their luminal concentration is low, a situation that may be analogous to that described in the rabbit [41]. It is important to take into account the possible variations in the pattern of distribution of the different amino acid transport systems along the intestine, since zonal differences have been described in different species, including the rat [41,42,43]; the lowest amino acid uptake rates in the rat being found in the duodenum and the highest in the jejunum [42,43]. Therefore, the utilization of whole intestine preparations must include a sampling of all these functionally different transport systems, hampering the interpretation of results, but allowing to take into account all the possible variables concerned.

The pattern of inhibition of sodium-dependent alanine uptake in *fa/fa* rats, may be affected by some additional differential factors with respect to lean rats. Genetically obese rats have a defective thermoregulatory system [44] that may induce hyperphagia to maintain its metabolic rate [45] with residual metabolic heat, which results in the massive accumulation of fat. Thus, in the *fa/fa* rat, excessive intake may elicit an adaptive response to excess amino acid absorption: first lowering the uptake ratio, then increasing the amount of amino acids not absorbed [7] and also increasing the production and elimination of urea [6].

From the results presented it can be deduced that obesity affects the uptake of L-alanine by brush-border membranes of rat small intestine, by varying the transport capacity – as indicated by the increase of the V_{max} in the cafeteria-fed group and the decrease in the genetic obese group – without any variation in the affinity for the substrate. The different pattern showed by obese groups, one (cafeteria-fed) with higher values than controls and the other (genetic obesity) with lower values than controls seem to indicate that these modifications may be due to changes in the activity in the transporter molecules as a response both to diet composition (cafeteria-fed) and genetic factors (genetic obesity). Under cafeteria diet feeding, the massive presence of a high lipids and/or the slight relative deficiency of protein in the diet [7] should be considered as a possible controlling element of the variability of the capacity of transport. This situation contrasts with that of genetically obese animals, which ingest the same type of diet as controls but tend to diminish their uptake capacity, perhaps as an adaptive response to hyperphagia. Finally, since the uptake by brush-border membrane vesicles alone cannot explain the increase in the efficiency of L-alanine uptake, the possible role of other molecular-environment factors should be taken into account.

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