Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum

M. TORRAS-LLORT, J. F. SORIANO-GARCÍA, R. FERRER, AND M. MORETÓ
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Torras-Llort, M., J. F. Soriano-García, R. Ferrer, and M. Moretó. Effect of a lysine-enriched diet on L-lysine transport by the brush-border membrane of the chicken jejunum. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R69–R75, 1998.—The influx of L-lysine into apical vesicles from the chicken jejunum occurs through two systems, one with low Michaelis constant (Km) and features of system b0,+ and the other with relatively high Km, for L-lysine and with properties of system y+. In the present study the effect of a lysine-enriched diet (Lys, containing 68 g L-lysine/kg dietary protein, control animals 48 g/kg) on L-lysine uptake through both transport systems was investigated. Results show that
1) lysine enrichment had no effect on either body weight or the efficiency of food utilization. 2) In Lys-fed animals, the mediated L-lysine influx was best fitted to the two-system model with y+ and b0,+ activity. 3) In the presence of an Na+ gradient, total L-lysine uptake is significantly higher in Lys-fed animals than in control birds (about 40% increase). 4) Lys diet increases Km,b0,+ 6-fold (KSCN gradient) and 12-fold (NaSCN gradient) and maximum velocity (Vmax) by 6- and 20-fold, respectively. The effects of Lys enrichment on the y+-like system are only observed on the Vmax and in the presence of an Na+ gradient (30% increase). 5) Na+ is involved in the activation of the transport process in the L-lys-fed chickens, but there is no correlation between external Na+ concentration and L-lysine influx. In conclusion, both b0,+ and y+-like transport systems are upregulated by dietary lysine but with different kinetic profiles; the high-capacity y+-like carrier shows a Vmax increase without changes in Km,n whereas the low-capacity b0,+ -like system shows an increase in Vmax as well as in the Km,system b0,+; system y+; adaptive regulation

AN ESSENTIAL AMINO ACID with high significance in chicken development and performance is L-lysine (8, 15, 16, 19). This amino acid is taken up by the small intestine of the chicken by mechanisms that have been recently described; in the apical membrane there are two carrier systems differing in their kinetic properties and in the way they interact with neutral amino acids and which have been identified as systems b0,+ and y+ (26). Similar transport systems have been characterized in human intestinal epithelial Caco-2 cells (25).

Some reports point out that L-lysine also has a role in the improvement of weight gain and food efficiency in birds when its content in the diet is increased (20). We wanted to test this hypothesis by feeding chickens with a lysine-enriched diet to see whether we could relate the L-lysine content of the diet to nutritional parameters and to the absorptive function of the small intestine and whether the eventual changes in the intestinal transport of L-lysine induced by the diet agree with Diamond’s adaptive regulation hypothesis. Ferraris and Diamond (10) claim that dietary constituents regulate their own transport, albeit the patterns of regulation may differ according to the role of the transported substrate in metabolism, i.e., energetic, essential, or both. Transport regulation by dietary content is attributed to an increase in the number of specific carriers (7, 9, 30) and not to unspecific factors, such as membrane permeability, epithelial surface area, or ionic gradient. We wanted to see to what extent the pattern of intestinal L-lysine transport by the chicken intestine bears out the postulates of the hypothesis in fully adapted birds.

METHODS

Animals and diets. One-day-old male Label chickens were housed in cages in controlled temperature and humidity conditions, with a 18:6-h light-dark schedule. Animals were fed ad libitum for 1 wk with a standard diet and then randomly divided into two groups and fed for 5 additional wk with either the same diet (control diet) containing 48 g L-lysine/kg dietary protein or a diet enriched with L-lysine (Lys diet) containing added L-lysine to reach a value of 68 g/kg dietary protein. Food consumption and body weight were measured twice weekly throughout the experiment. Diets were isonitrogenic (13 MJ/kg food) and formulated to contain 20% protein, 5.52% lipid, and 38.5% carbohydrate. The composition in essential amino acids was (g/kg diet) 5 dl-methionine, 9.8 L-isoleucine, 23 L-leucine, 8 L-threonine, 13.2 L-arginine, 2.1 L-tryptophan, 9.1 L-methionine-L-cysteine, and either 9.6 g/kg (control) or 13.6 g/kg (Lys diet) of L-lysine.

Preparation of brush-border membrane vesicles. Membranes were prepared from the jejunum of 6-wk-old chickens fed either control diet or Lys diet. Animals were killed in the morning by neck fracture, without previous starvation. The jejunum (from the end of the duodenal loop to the Meckel diverticulum) was removed, immediately flushed with ice-cold saline, opened lengthwise, frozen in liquid N2, and stored at −80°C.

Brush-border membranes were isolated by the Mg2+ precipitation method of Kessler et al. (13), and the vesicles were prepared as previously described (26). The composition of the isolation medium was 300 mmol/l mannitol, 0.1 mmol/l MgSO4, 7H2O, 0.02% LiN3, and 20 mmol/l N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-tris(hydroxymethyl)ammonomethane (Tris), pH 7.4. Vesicles were diluted to a final protein concentration of 10–20 mg/ml and then frozen and stored in liquid N2. Each isolation batch corresponds to the jejunum of a single chicken, and in RESULTS “n” indicates the number of chickens or membrane preparations.

Protein and enzyme assays. Sucrase, a brush-border marker enzyme, and Na+-K+-adenosinetriphosphatase (ATPase), a basolateral marker enzyme, were routinely assayed, according to Dahlqvist (3) and Del Castillo and Robinson (4), respectively, in both dietary treatments. Protein was determined using the Bio-Rad protein assay, with bovine serum albumin as standard.

Transport studies. L-Lysine uptake was assayed for time periods ranging from 1 s to 1 h at 37°C using the rapid-filtration technique previously described (26). The vesicles
were incubated under isotonic conditions (320 mosmol/l) in an incubation medium containing 100 mmol/l mannitol, 0.2 mmol/l MgSO₄·7H₂O, 0.02% LiN₃, and 20 mmol/l HEPES-Tris, pH 7.4, 100 mmol/l of a salt (NaSCN or KSCN), and the appropriate concentration of labeled L-lysine (5–10 µmol/l) or D-glucose (100 µmol/l) as substrates. In experiments requiring salt equilibration across the membrane, vesicles were preincubated for 30 min in 100 mmol/l of the salt before being added to the incubation medium. Experiments were performed in triplicate using vesicles from different batches.

Some of the results obtained with the standard (control) diet, designed to discover the properties of L-lysine uptake in the chicken, were the subject of a previous publication (26). These results have been included in the tables and figures when necessary to facilitate comparison with the results of the present article.

NEM treatment. The effect of N-ethylmaleimide (NEM) on 5 and 100 µmol/l L-lysine uptake was studied as previously described (26). Brush-border membrane vesicles (BBMV) were preincubated with 0.5 mmol/l NEM for 30 min at 25°C. To wash off the inhibitor, vesicles (either treated or not with NEM) were sedimented by high-speed centrifugation (12,000 g), and the pellets were resuspended in isolation medium. The resulting suspension was used for transport experiments. Nontreated vesicles ran parallel to NEM-treated vesicles in both diets.

Chemicals. L-[U-14C]lysine and D-[U-14C]glucose were obtained from New England Nuclear Research Products (Dreieich, Germany). Unlabeled reagents, including the lysine used for diet enrichment, were from Sigma Chemical (St. Louis, MO). Reagents used to determine enzyme activities were from Boehringer (Mannheim, Germany).

Transport equations. The kinetic parameters of L-[14C]lysine transport were determined by nonlinear regression analysis (Enzfitter, Biosoft, Cambridge, UK), according to the strategy described by Devés et al. (6). The approach is based on the use of the relative transport rate as the inhibition constant of b0,

\[
\frac{F}{V} \frac{1}{V_0} = \frac{1}{1 + (I/K_{b0}^\infty)} + \frac{1}{1 + (I/K_y^\infty)}
\]

(1)

where \(K_{b0}^\infty\) and \(K_y^\infty\) are the inhibition constants of \(b0^\infty\) and \(y^\infty\)-like systems, respectively. \(I\) is the concentration of unlabeled inhibitor, and \(F\) is the "permeability" ratio, which reflects the relative contribution of each system to the total influx, considering the substrate concentration (S) used in kinetic experiments.

\[
F = \frac{V_{max}b_0^\infty \cdot K_m y^\infty}{V_{max}y^\infty \cdot K_m b_0^\infty}
\]

(2)

To calculate maximum velocity \((V_{max})\) values we used Eq. 3. This equation describes transport through two systems in the presence of a competitive inhibitor (I) located at the cis side. The values of Michaelis constant \((K_m)\) and \(K_i\) were obtained from Eqs. 1 and 2:

\[
V = \frac{V_{max}b_0^\infty \cdot S}{K_m b_0^\infty \cdot (1 + I/K_{b0}^\infty)} + \frac{V_{max}y^\infty \cdot S}{K_m y^\infty \cdot (1 + I/K_y^\infty)} + S
\]

(3)

Data analysis. The data were reported as means ± SE. All data were compared using analysis of variance except for kinetic studies, where Student's t-test was used. In both cases \(P < 0.05\) was considered statistically significant.

RESULTS

Effect of diets on body weight. One-week-old chicks had a mean weight of 80.2 ± 2.0 g. After 5 wk the weight of the birds receiving the control diet was 1.14 ± 0.02 kg \((n = 41)\) and that of the lysine-enriched diet \((\text{Lys diet})\) was 1.17 ± 0.02 kg \((n = 44)\). These values indicate that there is no effect of a lysine-enriched diet on body weight. The efficiency of food utilization, expressed as gram weight gain divided by gram food intake, was also the same during the 5-wk period \((0.32 ± 0.02\) in control diet and 0.34 ± 0.01 in Lys diet).

Properties of BBMV. Vesicles prepared from the jejunum of both control and Lys diet-fed chickens showed similar sucrose and \(\text{Na}^+\text{–K}^+\)-ATPase enrichment factors (Table 1), indicating low basolateral membrane contamination. To check the functional properties of BBMV and to detect any dietary effect on monosaccharide transport, D-glucose (100 µmol/l) uptake was examined under zero-trans 100 mmol/l NaSCN or KSCN (intravesicular salt concentration equals 0 mmol/l and extravesicular salt concentration equals 100 mmol/l). Table 1 shows that vesicles behave as expected and that no differences were observed between the control diet and the Lys diet-fed animals. The values at equilibrium show no dietary effects on either vesicular volume (around 0.6 µl/mg protein) or D-glucose membrane binding.

Time course of L-[14C]lysine. Figure 1 shows time-dependent uptake of L-lysine (100 µmol/l), expressed as a percentage of the 60-min uptake value, in the presence of an inwardly directed \(\text{Na}^+\)-gradient (100 mmol/l NaSCN). The overshoot appears after 20 s of incubation:

Table 1. Enzyme activities and recoveries of BBMV and 100 µmol/l D-glucose uptake

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recovery</th>
<th>Specific Activity</th>
<th>Enrichment Factor</th>
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<tbody>
<tr>
<td><strong>Sucrase</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>34.1 ± 1.8</td>
<td>10.5 ± 0.6</td>
<td>10.8 ± 2.0</td>
</tr>
<tr>
<td>Lys fed</td>
<td>29.5 ± 4.5</td>
<td>9.5 ± 0.9</td>
<td>10.3 ± 1.8</td>
</tr>
<tr>
<td><strong>Na⁺–K⁺-ATPase</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.7 ± 0.35</td>
<td>0.4 ± 0.20</td>
<td>0.26 ± 0.10</td>
</tr>
<tr>
<td>Lys fed</td>
<td>0.8 ± 0.38</td>
<td>0.4 ± 0.16</td>
<td>0.22 ± 0.08</td>
</tr>
<tr>
<td>(5) s NaSCN</td>
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<tr>
<td>60 min NaSCN</td>
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<td>5 s KSCN</td>
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<tr>
<td>60 min KSCN</td>
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</table>

\(\text{D-glucose uptake, pmol/mg protein}\)

| Control      | 555 ± 41 | 56.7 ± 4.8 | 11.8 ± 2.4 | 61.0 ± 7.6 |
| Lys fed      | 498 ± 20 | 59.2 ± 2.2 | 15.4 ± 2.1 | 61.8 ± 8.0 |

Values are means ± SE; \(n = 5\) membrane preparations. Recovery is expressed as a percentage of total activity of original homogenate recovered in vesicle fraction. Specific activity is expressed in pmol·mg protein⁻¹·s⁻¹. Enrichment factor was calculated as ratio of specific activity of vesicles to that of homogenate. \(\text{D-glucose uptake (100 pmol/l D-[14C]glucose)}\) was determined under 100 mmol/l NaSCN or KSCN gradient. BBMV, brush-border membrane vesicles. No statistical differences were detected between diets \((P > 0.05)\).
and is higher in vesicles from Lys diet than from control birds. Experiments performed in the absence of Na$^+$ (KSCN gradient) showed no differences between diets (data not shown). Values obtained at equilibrium (about 80 pmol/mg protein) indicate no dietary effects on vesicular volume nor on L-lysine fixation to the membrane, either in the presence of Na$^+$ or K$^+$.

Kinetics of L-lysine transport. Kinetic parameters were calculated from 10 µmol/l L-[14C]lysine influx, under zero-trans NaSCN or KSCN gradients (100 mmol/l), in the presence of increasing unlabeled L-lysine or L-methionine concentrations (0–20 mmol/l) (Fig. 2). The kinetic analysis was performed from 3-s incubation data. In preliminary experiments, it was shown that L-lysine influx is linear for at least 5 s and that binding to the membrane is not modified by the thiocyanate salt, in either dietary treatment (data not shown).

The relative transport rates (L-[14C]lysine transport with and without unlabeled L-lysine or L-methionine) were assayed to fit to one, two, or three transport systems. The best fit was always obtained when the two-transport system model was considered, consistent with the presence in the chicken jejunum of $\gamma^+$ and $b_0^+$ activity, as previously reported (26).

Table 2 shows the inhibition constants calculated for L-methionine. There is no dietary effect on the $\gamma^+$-like system either in the presence or absence of Na$^+$. In contrast, a significant dietary effect was found for the $b_0^+$-like system, which consists in a reduction of $K_m$ and $V_{max}$ in Lys animals (NaSCN/KSCN was 1.9-fold in controls and 2.5-fold in Lys animals). The $b_0^+$ activity shows a significant dietary effect consisting in an increase both in $K_m$ and $V_{max}$. This effect is apparently inconsistent with the observed decrease in $K_i$ for L-methionine with lysine feeding because L-methionine is competing with L-lysine for the same transport systems. We can speculate that the diet-induced changes in the transport protein may have
ments were carried out in the presence of Na\(^{+}\) and NaSCN. Under a KSCN gradient was not affected by dietary Lys diet-fed chickens. These results indicate that L-lysine is not cotransported with Na\(^{+}\), neither in control nor in Lys-fed animals, but they strongly suggest an activation effect of Na\(^{+}\) on L-lysine influx related to dietary lysine.

Experiments in preequilibrated vesicles. In NaSCN preequilibrated vesicles (intravesicular NaSCN concentration equals extravascular NaSCN concentration equals 100 mmol/l) L-lysine uptake (10 µmol/l) was significantly higher in Lys animals (9.3 ± 0.2 pmol·mg protein\(^{-1}\)·s\(^{-1}\)) than in control chickens (7.0 ± 0.4 pmol·mg protein\(^{-1}\)·s\(^{-1}\)). This effect was not observed in vesicles equilibrated with KSCN (Lys 6.72 ± 0.5, control 6.0 ± 0.7 pmol·mg protein\(^{-1}\)·s\(^{-1}\)), indicating that Na\(^{+}\) “facilitates” L-lysine movement across the membrane. In both conditions no overshoot is observed and the equilibrium values (60 min) are not modified by diet or ionic composition.

BCH inhibition. Because our results clearly show that cis Na\(^{+}\) has a stimulatory effect on L-lysine uptake, we wanted to prove the existence of Na\(^{+}\)-dependent transport for cationic and neutral amino acids (B\(^{0,+}\)-type system). B\(^{0,+}\) activity differs from B\(^{\lambda,+}\) activity in a different effect on the interaction of the protein with either charged or dipolar amino acids.

This group of experiments also indicate that the lysine-enriched diet modifies the Na\(^{+}\) sensitivity of both B\(^{\lambda,+}\)- and y\(^{-}\)-like systems (for B\(^{\lambda,+}\): KSCN gradient, 6- to 7-fold increase for both constants; NaSCN gradient, 12-fold increase in \(K_m\) and 20-fold increase in \(V_{\text{max}}\)).

Figure 2 shows that uptake of 10 µmol/l L-lysine under a KSCN gradient was not affected by dietary lysine (control 31.0 ± 4.5 pmol L-lysine·mg protein\(^{-1}\)·s\(^{-1}\) and Lys 29.3 ± 2.2). However, when the experiments were carried out in the presence of Na\(^{+}\), total uptake in Lys animals (40.8 ± 3.1) was higher than in control birds (30.1 ± 4.2).

NEM treatment. To validate the kinetic effects reported, experiments were performed in NEM-pre-treated vesicles to inhibit the y\(^{-}\)-activity (5). In these conditions \(V_{\text{max}}\) was calculated from \(K_m\) values and from 5 µmol/l L-[\(^{14}\)C]lysine influx in the absence of unlabeled amino acid. The \(V_{\text{max}}\) (expressed in pmol·mg protein\(^{-1}\)·s\(^{-1}\)) was still higher in Lys-fed animals than in control animals, and an Na\(^{+}\) effect was also observed in the Lys diet (Lys diet, KSCN 30.0 ± 2.15 and NaSCN 70.8 ± 14.2; control diet, KSCN 5.5 ± 0.67 and NaSCN 4.7 ± 0.6). Moreover, experiments performed in the presence of NEM, with L-lysine at a much higher concentration (100 µmol/l), also show an effect of external Na\(^{+}\) (KSCN 30.9 ± 1.1 and NaSCN 60.1 ± 3.4 pmol·mg protein\(^{-1}\)·s\(^{-1}\)), which supports the kinetic predictions for the b\(^{0,+}\)-like system in Lys-fed animals.

Effect of Na\(^{+}\) gradient. As shown before, Na\(^{+}\) is relevant in the uptake process of L-lysine by the brush border. To investigate the possible existence of an Na\(^{+}\)-dependent component in Lys-fed animals, initial rates of L-lysine transport were determined in the presence of increasing zero-trans NaSCN gradients, ranging from 0 to 100 mmol/l. Figure 3 shows that Na\(^{+}\) has a stimulating effect, which is maximal at already low Na\(^{+}\) concentrations (0.25 mmol/l). Therefore, there is no correlation between the external Na\(^{+}\) concentration and the rate of L-lysine transport in the Lys diet-fed chickens. These results indicate that L-lysine is not cotransported with Na\(^{+}\), neither in control nor in Lys-fed animals, but they strongly suggest an activation effect of Na\(^{+}\) on L-lysine influx related to dietary lysine.

Because our results clearly show that cis Na\(^{+}\) has a stimulatory effect on L-lysine uptake, we wanted to prove the existence of Na\(^{+}\)-dependent transport for cationic and neutral amino acids (B\(^{0,+}\)-type system). B\(^{0,+}\) activity differs from B\(^{\lambda,+}\) activity in.

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**Table 2.** \(K_m\) in presence of unlabeled increasing L-methionine concentrations of 10 µmol/l L-[\(^{14}\)C]lysine

<table>
<thead>
<tr>
<th></th>
<th>KSCN</th>
<th>NaSCN</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Lys diet</td>
</tr>
<tr>
<td>(K_m) (b^{0,+}), µmol/l</td>
<td>55.0 ± 8.4</td>
<td>29.4 ± 5.1(</td>
</tr>
<tr>
<td>(K_m) (y^{-}), µmol/l</td>
<td>3.4 ± 0.9</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>(V_{\text{max}})</td>
<td>16.6 ± 0.12</td>
<td>19.0 ± 0.3</td>
</tr>
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</table>

Values are means ± SE; n = 4 or 5 membrane preparations. Kinetic constants were determined under NaSCN or KSCN zero-trans gradient. Michaelis constant \((K_m)\) was calculated by nonlinear regression analysis from values of relative rates \((v/v_0)\) and with the permeability ratio calculated in Table 2 taken as fixed value. Maximal velocity \((V_{\text{max}})\) values (expressed as pmol L-lysine·mg protein\(^{-1}\)·s\(^{-1}\)) were calculated from total influx values as indicated in METHODS (Eq. 3). Statistical analysis showed significant differences between the kinetic constants \(|t|\) among diets and in \(|t|\) presence or absence of NaSCN (P < 0.05).

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**Table 3.** Kinetic constants of L-lysine transport

<table>
<thead>
<tr>
<th></th>
<th>KSCN</th>
<th>NaSCN</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Lys diet</td>
</tr>
<tr>
<td>(K_m) (b^{0,+}), µmol/l</td>
<td>2.7 ± 0.62</td>
<td>17.7 ± 2.0(</td>
</tr>
<tr>
<td>(V_{\text{max}}) (b^{0,+}), pmol/l</td>
<td>5.16 ± 0.9</td>
<td>34.2 ± 1.8(</td>
</tr>
<tr>
<td>(K_m) (y^{-}), µmol/l</td>
<td>0.13 ± 0.02</td>
<td>0.16 ± 0.034</td>
</tr>
<tr>
<td>(V_{\text{max}}) (y^{-}), pmol/l</td>
<td>155.3 ± 7.0</td>
<td>161.2 ± 11.0</td>
</tr>
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</table>

Values are means ± SE; n = 4 or 5 membrane preparations. Kinetic constants were determined under NaSCN or KSCN zero-trans gradient. Michaelis constant \((K_m)\) was calculated by nonlinear regression analysis from values of relative rates \((v/v_0)\) and with the permeability ratio calculated in Table 2 taken as fixed value. Maximal velocity \((V_{\text{max}})\) values (expressed as pmol L-lysine·mg protein\(^{-1}\)·s\(^{-1}\)) were calculated from total influx values as indicated in METHODS (Eq. 3). Statistical analysis showed significant differences between the kinetic constants \(|t|\) among diets and in \(|t|\) presence or absence of NaSCN (P < 0.05).
DISCUSSION

Dietary treatment. Following the recommendations of the National Research Council (22) for broilers, a control diet containing 20% protein and 0.96% L-lysine was formulated to assure that the amount of L-lysine would not be limiting for animal growth and performance. Because Morris et al. (20) found an improvement in body weight gain and in the efficiency of food utilization by increasing L-lysine content from 0.94 to 1.27%, a lysine-enriched diet was designed containing 1.36% L-lysine, i.e., a 42% increase relative to the control diet. In these conditions, the dietary effects observed should be attributed to either diet supplementation of this specific amino acid or to an effect resulting from the different L-lysine/protein ratio between control and Lys group (0.048) and the Lys group (0.068).

Our results show, however, that the protocol employed in the present study had no influence on either chicken growth or on food utilization. This was not totally unexpected because other laboratories have reported controversial results in lysine-supplemented animals; for example, Ledergerber et al. (15) have shown that chickens selected for rapid growth are less sensitive to L-lysine levels in the food than lean-line birds.

Although L-lysine enrichment was not reflected in conventional nutritional parameters, the higher luminal L-lysine concentration was found to exert a significant effect on specific transport systems that participate in lysine absorption.

Properties of L-lysine transport. The y⁺- and b⁰⁺⁺-like systems are involved in L-lysine transport in the chicken jejunum. System b⁰⁺⁺ shows low $K_m$ and low capacity for L-lysine transport, whereas $y^+$ has higher $K_m$ and a relatively high transport capacity (26). In addition, both transport systems are able to interact with L-methionine albeit with different inhibition constants ($K_{iy^+}$ is much higher than $K_{ib^{0+}}$, Table 2). Recent experiments carried out in these membranes, using L-methionine as substrate (23), strongly indicate the presence of only two pathways sensitive to L-lysine inhibition and with kinetic properties closely similar to those described for L-lysine ($K_m$ of L-methionine similar to its $K_i$, against L-lysine and vice versa).

In the chicken, the contribution of both systems to mediated transport in control conditions (calculated for a 10 µmol/l L-lysine concentration) is 12% for $b^{0+}$ and 88% for $y^+$. This profile differs from that found in human epithelial Caco-2 cells, where $b^{0+}$ and $y^+$ contribute 64 and 36%, respectively (25).

L-lysine transport was found to be sensitive to membrane potential, although the effects are mainly seen in relatively long-term (20-s) incubations, probably indicating a slow movement of L-lysine across the membrane (26). Such a slow rate of influx is enhanced by external Na⁺ as indicated by the significant increase in $V_{max_y^+}$. This effect can be attributed to a rise in the inside negative potential due to the different membrane permeability to Na⁺ and K⁺. The effect of Na⁺ is further potentiated by the lysine-enriched diet.

A lysine-enriched diet increases the intestinal L-lysine influx in the presence of Na⁺ because the lys diet enhances system $y^+$-like sensitivity to Na⁺ and induces Na⁺ sensitivity in the $b^{0+}$ transport activity. Because system $b^{0+}$ does not seem to be greatly affected by the membrane potential, the role of Na⁺ can be better interpreted as an effect on the efficiency of the transport process. The possibility of a change in the membrane permeability to Na⁺ in Lys-fed animals was rejected because Na⁺-dependent, β-glucose uptake, a process that is highly sensitive to membrane potential (21), was not affected by lysine enrichment.

In the absence of Na⁺ time-dependent L-lysine transport is not modified by the dietary treatment, and the kinetic constants of the $y^+$-like system are not affected by lysine enrichment either. The $b^{0+}$-like system shows a six- to sevenfold increase in $V_{max}$ and $K_m$; these changes may explain why dietary lysine has little effect on total L-lysine influx when K⁺ substitutes Na⁺. Under a Na⁺ gradient, Lys diet adaptation has a much greater effect on the kinetic constants of the $b^{0+}$- than of the $y^+$-like system. In these conditions, the change in the relative contribution of each system to L-lysine uptake (manifested by the significant increase in $F$), confirms the increase in the contribution of the $b^{0+}$-like system. This dietary effect, in addition to the dietary increase in the $V_{max_y^+}$, results in a higher total L-lysine influx. It is worth noting that the effects of Lys diet are...
also observed in preequilibrated vesicles, in the absence of ionic or membrane potential gradients.

The effects of the Lys diet on L-lysine transport are fully manifested with Na⁺ present in the incubation medium, suggesting that dietary lysine stimulates a regulated Na⁺-dependent transport system with high selectivity for cationic amino acids, similar to what was reported by Wolfrem et al. (29) in rats fed a high-protein diet. This would be another example of paired transport systems for cationic amino acids that have similar substrate selectivity but differ only in their Na⁺ dependence. The possible existence of a B⁰,⁰⁺-like transport activity was studied using BCH as specific inhibitor (28). The results show that this analog inhibits lysine uptake to a similar extent (about 30%) both in the presence and in the absence of Na⁺, thus weakening the existence of a B⁰,⁺⁺ carrier. The inhibition observed with BCH is consistent with the results of Bertran et al. (2) showing an interaction of high BCH concentrations with the b⁰,⁺⁺ activity in Xenopus oocytes.

Na⁺ is not required for the activation of L-lysine transport across b⁰,⁺⁺ or y⁺⁺-like systems, but influx in the presence of Na⁺ is greater than in its absence. This stimulatory effect of Na⁺ is already seen at low Na⁺ concentrations and there is no correlation between Na⁺ concentration and L-lysine influx (i.e., L-lysine influx is “Na⁺ independent”). Therefore, Na⁺ may have an activating role, resulting in an effect on Vₘₐₓ, a “velocity effect” (14, 17, 24), but need not necessarily be transported into the vesicles together with the amino acid. Similar observations were made by Mircheff et al. (18) in rabbit renal brush-border vesicles: an inwardly directed Na⁺ gradient accelerates L-lysine uptake compared with a K⁺ gradient by a process that is described to be Na⁺ independent. In the experiments using NaSCN preequilibrated vesicles, L-lysine influx was higher in Lys animals than in control chickens (there was no effect in vesicles preequilibrated with KSCN). Because in these conditions no ionic diffusion potentials are expected and the only gradient is the chemical gradient, the results indicate that Na⁺ exerts some facilitation on the transport process that is membrane potential independent.

It is thus concluded that the effects of L-lysine supplementation can be explained by changes in the already described transport systems rather than the appearance of new transporters. It is also concluded that transport of L-lysine does not appear to absolutely depend on Na⁺ and that lysine supplementation stimulates the role of Na⁺ in the activation of the transport process.

Adaptive regulation. The intestinal absorption of nutrients is regulated, among other factors, by dietary substrate levels. According to the adaptive regulation hypothesis (9–11) a nutrient carrier is repressed when its biosynthetic costs exceed the benefit it provides and vice versa. For example, the transport of α-glucose and α-fructose, which are nonessential nontoxic nutrients that can be used as a source of energy, is upregulated by luminal sugars. However, essential amino acids such as L-lysine may induce a different pattern, depending on their degree of toxicity and whether their transport is shared with other amino acids.

Karasov et al. (12) fed mice with a diet supplemented with 6% L-lysine, and they observed that dietary lysine induces a 19% stimulation of normalized L-lysine uptake; because experiments were done using high substrate external concentrations, the increase was ascribed to an effect on the number of carriers. Growth rates and body weight were, however, lower than animals fed nonessential amino acids. No significant effects of L-lysine were observed by Karasov et al. (12) on intestinal morphometric parameters. This is consistent with preliminary results of Amat et al. (1) showing no changes in nominal surface area in the small intestine of Lys-fed chickens.

From calculations based on the kinetic constants of Table 3 it has been estimated that chickens fed the Lys diet show about a 50% increase in total influx with Na⁺ present in the lumen. At high luminal lysine concentrations (e.g., 10 mmol/l) increased transport is due to the 2-fold increase in b⁰,⁺⁺ activity and to the 36% increase across y⁺⁺-like system; however, because the y⁺⁺ system is a high-capacity transport mechanism, its contribution to the increased amount of L-lysine taken up is much higher (64%) than that of b⁰,⁺⁺-like system (contributes 36%). At low substrate concentrations (1–10 µmol/l), however, the contribution of system b⁰,⁺⁺ to increased uptake is higher (88%) than the contribution of system y⁺⁺ (only 12%).

The high-capacity y⁺⁺-like system behaves as predicted by the adaptive regulation hypothesis; it shows no variations in Kₘ and a significant increase in Vₘₐₓ. It is a typical induction in the activity of carriers in response to the increased luminal lysine concentration. The low-capacity system identified as b⁰,⁺⁺ shows increased Vₘₐₓ and Kₘ for the substrate, although the L-lysine transport rates are increased at both low (2–3 fold) and high (30-fold) substrate concentrations. The change in Kₘ would not support the hypothesis, but it must be considered that the Kₘ values of the b⁰,⁺⁺ system may be overestimated in our experimental conditions.

In conclusion, dietary lysine supplementation results in an increased L-lysine transport capacity by the chicken intestine as a result of both upregulation of existing carriers and an increased Na⁺-sensitivity in the activation process.

Perspectives

The transport of cationic amino acids across biological membranes can be mediated by a number of carriers, namely y⁺⁺, y', L, b⁰⁺⁺, and B⁰⁺⁺. In the present paper and in a previous one (26) we have confirmed the presence in the intestine of a b⁰⁺⁺ (but not B⁰⁺⁺) activity, and our results strongly indicate that an activity resembling y⁺⁺ is functionally present in the luminal brush-border membrane of the chicken.

It is interesting to note that the intestinal systems involved in L-lysine uptake interact with neutral amino acids and also that Na⁺ has an activating role in
transport apparently without being cotransported with the substrate, and both features need further investigation. It will also be interesting to see whether dietary supplementation of the chickens with L-methionine, an essential amino acid with some degree of toxicity, can downregulate L-methionine uptake by the intestine of this animal species, as predicted by the adaptive regulation hypothesis.

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