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System $b^{0,+}$-mediated regulation of lysine transport in Caco-2 human intestinal cells

Short Communication

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Summary. We investigated whether lysine transport would be subject to adaptive regulation in Caco-2 human intestinal cells. The activity of Lys transport in Caco-2 cells decreased with increasing incubation time with 10 mM Lys. Among the two systems involved in Lys transport, the system $b^{0,+}$ component was greatly decreased by incubating cells with 10 mM Lys, whereas the system y^+ component did not change. These results suggest that system $b^{0,+}$ mainly contributes to the adaptive regulation of Lys transport in Caco-2 cells.

Keywords: Amino acids – Lysine – Transporter – Caco-2 – Adaptive regulation

Introduction

Amino acids are understood to be transported via a specific system. Amino acid transport systems consist mainly of the carrier protein (transporter) itself and of its regulatory protein, some of the carrier proteins such as CATs having been identified and its regulation investigated at a molecular level (McGivan and Pastor-Anglada, 1994; Malandro and Kilberg, 1996; Closs, 1996; MacLeod and Kakuda, 1996).

The transport of a single amino acid, especially a neutral or cationic amino acid, is usually mediated by plural transport systems. For example, the transport of cationic amino acids in the small intestine is mediated by systems $b^{0,+}$ and y^+ as has recently been reported by Thwaites et al. (1996). They characterized the transepithelial and cellular lysine (Lys) transport, and showed that Lys transport was mediated mainly by systems $b^{0,+}$ and y^+, together with a component of very low affinity. The relative contribution of $b^{0,+}$ and y^+ to total Lys transport in the Caco-2 human intestinal cultured cell line was estimated to be 47% and 27%, respectively.

Such amino acid transport systems as A, L, N_m and X_{AG}^- have been reported to be subject to adaptive regulation (Moreno et al., 1985; Tadros et al., 1993; Low et al., 1996; McGivan et al., 1996). With this regulation, cells maintain their physiological states, adapting to the extracellular conditions. The adaptive regulation of amino acid transport in the small intestine is of great importance, because the supply of amino acids to the animal body is largely dependent on the intestinal absorption. However, this type of regulation has not yet been observed in the small intestine.

The present study was undertaken to reveal whether intestinal Lys transport would be subject to adaptive regulation. The regulatability of Lys transport was investigated by using the Caco-2 intestinal cell line which differentiates spontaneously and exhibits various enterocytic characteristics (Hidalgo et al., 1989). We found that the Lys transport in Caco-2 cells was down-regulated by culturing the cells with a Lys-containing medium and that system $b^{0,+}$ was solely responsible for this down-regulation.

Materials and methods

Materials

The Caco-2 cell line was obtained from American Type Culture Collection (Rockville, MD, U.S.A.). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan), and fetal calf serum (FCS) was from Gibco (Gaithersburg, MD, U.S.A.). [4,5-^{3}H] Lysine monohydrochloride (specific radioactivity of 85.0 Ci/mmol) was purchased from Amersham (Little Chalfont, England). All the other chemicals used were of reagent grade.

Cell culture

Caco-2 cells were cultured with a culture medium consisting of DMEM, 10% FCS, 1% non-essential amino acids, 2% glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin and an appropriate amount of sodium bicarbonate. The cells were incubated at 37°C under a humidified atmosphere of 5% CO_2 in air. For uptake experiments, Caco-2 cells were cultured in 24-well plates that had been precoated with collagen at a density of 1.4×10^5 cells/well. Cell monolayers for these uptake experiments were used after 14 days of culture.

Uptake experiments

[^{3}H] Lys uptake experiments were performed in the absence (total uptake) or presence (non-specific uptake) of 50 mM unlabelled Lys, allowing the specific uptake to be calculated by subtraction.

The Caco-2 monolayers were washed twice with 700 μl of PBS for 5 min and once with 300 μl of an uptake buffer of a sodium-free Hank's balanced salt solution (HBSS) for 15 min. Sodium-free HBSS was prepared by replacing NaCl with choline chloride and adjusting the pH value to 7.4 with KOH. The cells were incubated at 37°C for 10 min with 0.3 μCi of [^{3}H] lysine monohydrochloride in 300 μl of the uptake buffer (11.7 nM) with or without excess (50 mM) lysine monohydrochloride. After washing carefully three times with 700 μl of ice-cold PBS containing 0.05% sodium azide, 250 μl of 0.1% TritonX-100 was added to each well to render the cells soluble. The tritium content of each monolayer was finally determined with an LSC 5,100 liquid scintillation analyzer (Aloka, Tokyo, Japan).

Results and discussion

After culturing the Caco-2 cells for 14 days, the medium was changed to the one containing 10mM Lys. The monolayers were incubated for 12, 24, 36, or 48 hours, and the uptake experiments were then performed. As shown in Fig. 1, the transport activity decreased as the time of incubation with 10mM Lys was increased, the value decreasing by up to 55% of its initial value. This suggests that some of the transport systems for Lys were down-regulated in the Caco-2 cells by culturing with 10mM Lys.

Lys transport in Caco-2 has been reported by Thwaites et al. (1996) to be mainly mediated by $b^{0,+}$, y^+, and a non-saturable component. We therefore investigated which system was down-regulated by incubating the cells with 10mM Lys. The Thwaites method was applied to discriminate the total Lys uptake by the component of each amino acid transport system. Figure 2 shows that the Lys uptake inhibited by 10mM homoserine (HS) under a sodium-free condition (the component of system $b^{0,+}$) was greatly decreased as the time of incubation with 10mM Lys was increased, whereas the uptake not inhibited by 10mM HS (the component of system y^+) was not changed. These results indicate that the down-regulation of system $b^{0,+}$ contributed to the Lys-induced down-regulation of Lys uptake in Caco-2 cells.

The adaptive regulation of Lys transport has not previously been reported. The present result is the first to indicate that Lys transport is subject to adaptive regulation and plural Lys transport systems are individually regulated under a Lys-rich condition.

Independent changes in the activities of more than two amino acid transport systems have been reported in vascular smooth muscle cells (VSMC). In VSMC, arginine transport is mainly performed by system y^+ and system $B^{0,+}$.

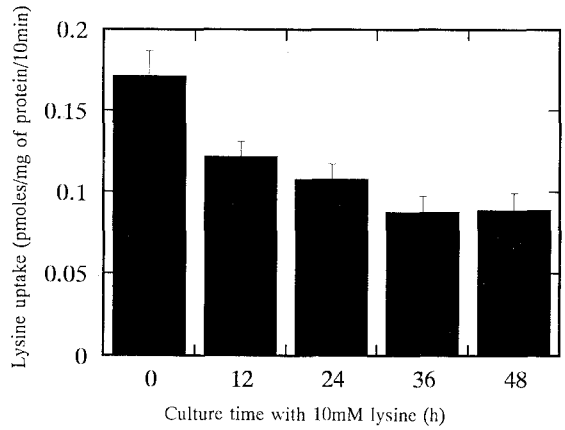

Fig. 1. Effect of culture time with 10mM Lys on the Lys uptake by Caco-2 monolayers. Cells were precultured in a medium containing Lys for various times, uptake experiments then being performed as described under "Materials and methods." Each value is the mean ± SEM (n = 4)

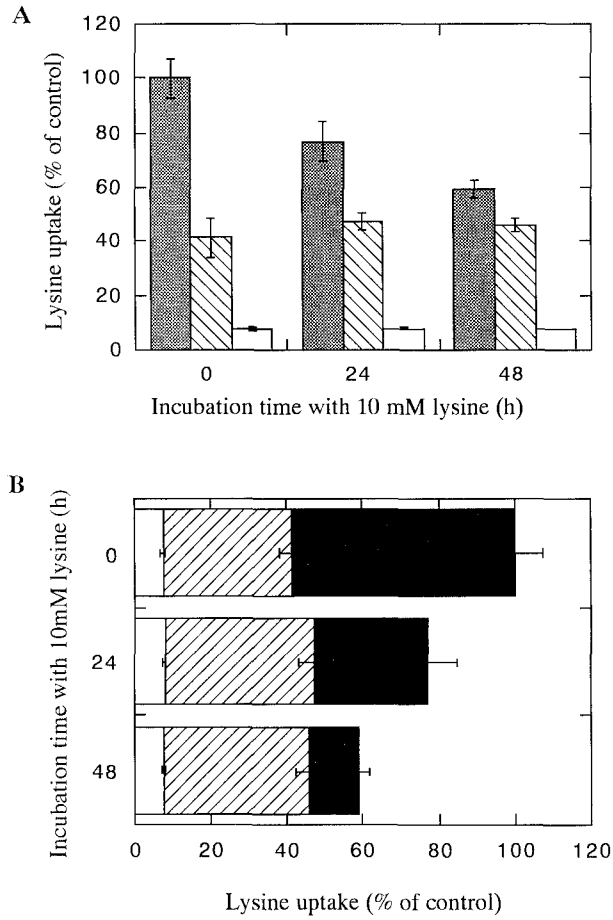

Fig. 2. Relative contribution of systems $b^{0,+}$ and y^+ to the Lys-induced down-regulation by Caco-2 monolayers. **A** Cells were precultured several times with 10 mM Lys and uptake experiments were performed in the absence (total uptake, ▨) or presence of 10 mM homoserine (▧), and in the presence of both 10 mM homoserine and 10 mM Lys (□). **B** The system $b^{0,+}$ component (■) was calculated by subtracting the uptake in the presence of 10 mM homoserine from the total uptake. The system y^+ component (▨) was calculated by subtracting the uptake in the presence of both 10 mM homoserine and 10 mM Lys from the uptake in the presence of 10 mM homoserine alone. Each value is the mean ± SEM (n = 4)

System $B^{0,+}$ is a sodium-dependent, neutral and dibasic transport system whose substrates are similar to those of system $b^{0,+}$. Rivera-Correa et al. (1996) have reported that arginine transport via the system $B^{0,+}$ in VSMC was decreased to only 6% by treating the cells with angiotensin II, whereas arginine transport via system y^+ was not changed at all. Although this down-regulation was not adaptive, this phenomenon resembles to our present results in terms of low responsiveness of system y^+ and high responsiveness of systems $b^{0,+}$ and $B^{0,+}$ to the extracellular conditions.

An excess supply of Lys, Met, or Val is known to inhibit the growth of rats (Russell et al., 1952). Therefore, it may be reasonable to assume that Lys

transport would be down-regulated under a Lys-rich condition in the small intestine, suppressing the absorption of excessive Lys by the whole body. However, if all of the Lys transport systems have the same regulatory mechanism against a Lys-rich condition, the absorption of Lys would be totally stopped. In order to avoid such a situation, each amino acid transport system may have an individually differing adaptive response; e.g., system $b^{0,+}$ would be regulatable and system y^+ stable, in response to an extensive change in Lys concentration.

The D2H protein, one of the rBAT protein family, is likely to participate in the transport of amino acids via system $b^{0,+}$ (Malandro and Kilberg, 1996). It is now clear that mutations of the rBAT-related protein are associated with the clinical condition of cystinuria, in which the renal (and intestinal) transport of dibasic amino acids and cystine is disordered (Calonge et al., 1994; Palacin et al., 1996). The hydrophobicity profile of D2H has suggested that the protein contains either one or four transmembrane domains (Lee et al., 1993; Malandro and Kilberg, 1996). Thus, D2H is likely to participate in system $b^{0,+}$ as a regulatory membrane protein, but not to be the transporter itself. To evaluate whether this down-regulation would be accompanied by changes in D2H at the transcriptional level, we performed a northern analysis of D2H, using PCR products of D2H. This northern analysis applied 3μg of poly(A)$^+$ RNA purified from total RNA which had been extracted from Caco-2 cells cultured with 10mM Lys for 24 or 48 hours. The result showed that the expression level of D2H was not altered at all (data not shown), indicating that no Lys-induced down-regulation of system $b^{0,+}$ occurred, at least at the transcriptional level of D2H. This down-regulation may therefore have been due to the post-transcriptional level of D2H, or to regulation of the transporter itself or of other unknown regulatory factors involved in system $b^{0,+}$. In order to elucidate the mechanism for this regulation, it is necessary to identify the transporter itself which participates in system $b^{0,+}$.

In conclusion, the present study shows that the activity of Lys transport in human intestinal Caco-2 cells was subject to adaptive regulation and that only system $b^{0,+}$ showed an adaptive response. We are now in the process of investigating the intracellular mechanism for this regulation by using the Caco-2 cell line, a well-defined *in vitro* model of human intestinal epithelial cells.

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