Diamines interfere with the transport of L-ornithine in Ehrlich-cell plasma-membrane vesicles

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1. L-Ornithine transport by plasma-membrane vesicles isolated from Ehrlich cells is Na⁺-independent and shows a saturable and a diffusional component. 2. Putrescine, histamine, 5-hydroxytryptamine and 2,3-diaminopropane at 55 μ M concentration significantly inhibit 0.5 mM-L-ornithine transport, at least for the first 10 min of incubation. 3. There is a *trans*-stimulatory effect of putrescine on L-ornithine transport.

INTRODUCTION

In a variety of cell types there is a single transport system that accounts for bidirectional transmembrane fluxes of cationic amino acids [1]. This system, named y⁺, was first described by Christensen [2] as a specific transport system for lysine, although later it was characterized as a general transport system for cationic amino acids [3,4]. System y⁺ is characterized as Na⁺-independent and pH-insensitive [1]; it is also strongly *trans*-stimulated and *cis*-inhibited by cationic amino acids [1,5,6]. In the presence of Na⁺, this system may interact with moderate affinity with neutral amino acids [2,3,7]. It is noteworthy that in some cell types, a Na⁺-dependent transport system, named Y⁺, contributes to the uptake of cationic amino acids [1,8].

Ornithine is a non-essential cationic amino acid that plays an important role as an intermediate in the urea cycle and as a precursor for polyamine biosynthesis [9,10]. In a previous paper, a net flux of ornithine from host tissues to tumour cells in growing Ehrlich ascites carcinoma was demonstrated [11]. When Ehrlich cells were continuously incubated in the presence of ornithine and polyamines, there was a decrease in the intracellular concentration of ornithine [12]; this fact suggests that these amines interfere with the ornithine-transport system. The present paper reports evidence for an inhibitory effect of diamines on the transport of ornithine in plasma membranes isolated from Ehrlich cells by two-phase compartmentation.

MATERIALS AND METHODS

Highly pure plasma-membrane vesicles were isolated from Ehrlich cells by two-phase compartmentation with poly(ethylene glycol) 3350 and dextran 500, and they were stored as described elsewhere [13]. Proteins were measured by the dye-binding procedure described by Bradford [14]. Membrane preparations stored in 15% (v/v) dimethyl sulphoxide were quickly thawed. To remove the dimethyl sulphoxide, the membranes were precipitated by adding 0.1 M-NaCl (final concn.), and centrifuged at 16300 g for 10 min. The pellet was suspended in 5 mM-Hepes buffer/0.1 M-KCl, pH 7.4, and centrifuged once more. The final pellet was suspended in the same buffer at 6 mg of protein/ml.

Transport experiments were initiated by mixing, in a 1:4 ratio, the membrane suspension with buffer (125 mM-NaSCN, 5 mM-Hepes, pH 7.4) in the presence of 0.5 mM (final concn.) L-[1-¹⁴C]ornithine (11 d.p.m./pmol), L-[G-³H]glutamine (110 d.p.m./pmol) or D-[U-¹⁴C]glucose. At each assay time, a 50 μ l sample was filtered through a glass-fibre filter (Whatman GF/C), and rapidly washed with 10 ml of ice-cold stop solution (0.1 M-

NaCl/5 mM-Hepes buffer, pH 7.4). Transport experiments were carried out at room temperature. The filters were dried at 60 °C and then placed into scintillation vials containing 4 ml of Aquasol-2, and radioactivity was determined in a liquid-scintillation counter. All the measurements were carried out at least in triplicate and were corrected for non-specific binding, calculated from the radioactivity retained in the filters at zero time.

Kinetic parameters were determined by non-linear regression analysis. The equation used was $v = V_{max} \cdot [S]/(K_m + [S]) + [S] \cdot K_d$. This equation describes the transport process as the sum of a single saturable system plus a non-saturable component formally indistinguishable from diffusion.

RESULTS AND DISCUSSION

There was no significant difference in the rate of transport of L-ornithine when plasma-membrane vesicles isolated from Ehrlich cells were incubated in equiosmolar concentrations of NaSCN, KSCN or mannitol (results not shown). This fact reveals that Ehrlich cells are deficient in Na⁺-dependent transport system Y⁺, such as that described for intestine [15]. To maintain the osmolarity of the medium and the same ionic conditions, all the experiments shown in this paper were carried out with externally added 100 mM-NaSCN (final concn.). The anion thiocyanate easily diffuses across the phospholipid bilayer, increasing the membrane potential and favouring the Na⁺-dependent systems tested below. Fig. 1 shows the time course of L-ornithine transport, which seems to reach a maximum after 10 min. The shape of the uptake curve (Fig. 1) suggests that

Fig. 1. Time course of L-ornithine transport in plasma-membrane vesicles isolated from Ehrlich cells

Vesicles were incubated in the presence of 0.5 mM-L-[1-¹⁴C]ornithine, and transport was carried out as described in the Materials and methods section. A typical result is presented.

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Table 1. Inhibitory effect of putrescine on L-ornithine transport by Ehrlich-cell plasma-membrane vesicles

Transport experiments were carried out with 0.5 mM-L-[1-¹⁴C]ornithine in the absence or presence of 55 μ M-putrescine as described in the Materials and methods section. The values are means $\pm$ S.D. for at least four determinations and are given as percentages of the transport measured in the absence of putrescine. All values except that for 30 min are significantly different ($P < 0.05$) from the corresponding control values.

Time (min)	Transport (%)
0.5	47 $\pm$ 12
1	78 $\pm$ 21
2	67 $\pm$ 19
10	70 $\pm$ 19
30	99 $\pm$ 23

Fig. 2. Kinetics of L-ornithine transport (a) and kinetics of inhibition by putrescine (b)

(a) Transport was determined at different L-ornithine concentrations in 100 mM-NaSCN. Transport was stopped after 30 s. Corrected values are represented. (b) Transport was determined at 0.5 mM-L-ornithine (●) and 50 μ M-L-ornithine (○) in the presence of different putrescine concentrations in 100 mM-NaSCN. Transport was stopped after 2 min. Corrected values for the saturable component of the transport are represented.

the equilibrium between external and internal concentrations of ornithine is reached after this time.

Interference with cationic amino acid transport caused by neutral amino acids in the presence of Na⁺ has been previously reported [3,4,7]. However, to our knowledge, no interference by polyamines with transport system y⁺ has been reported. Matés *et al.* [16] have shown that 1,4-diamines (at micromolar concentrations) inhibit the ornithine decarboxylase activity induced by 0.5 mM-L-ornithine in perfused tumour cells [16]. Furthermore, the presence of 55 μ M diamines in a perfusion medium containing 0.5 mM-L-ornithine decreased intracellular L-ornithine [12]; these results led us to try to demonstrate an interference of diamines with L-ornithine transport. Table 1 shows the inhibitory effect of 55 μ M-putrescine on transport of 0.5 mM-L-ornithine by Ehrlich-

Fig. 3. Time course of L-glutamine transport in the absence and in the presence of putrescine

The transport of 0.5 mM-L-[G-³H]glutamine by plasma-membrane vesicles was measured in the absence (○) or the presence (●) of 55 μ M-putrescine.

cell vesicles. This inhibition is significant, at least during the first 10 min, when ornithine concentration is not yet equilibrated inside and outside vesicles.

The kinetic curve of L-ornithine transport (Fig. 2a) was adjusted to a best-fit curve, giving apparent $K_m = 0.32$ mM, $K_d = 136$ pmol/mg of protein per mM, and $V_{max} = 400$ pmol/mg of protein per 2 min. In the presence of various concentrations of putrescine, similar inhibition curves were obtained for the saturable component in the transport of 50 μ M- and 0.5 mM-L-ornithine (Fig. 2b). These curves are similar to previously reported inhibition curves of cationic amino acids on transport system y⁺ [1,3,4,6]. When 50 μ M-L-ornithine was used, 5 μ M-putrescine gave an apparent inhibition of 58%; with 0.5 mM-L-ornithine, 50 μ M-putrescine gave an apparent inhibition of 28%. However, experimental data could not be adjusted to a theoretical model of Michaelis reversible inhibition. Metabolite interferences with transport are complex processes. Even for previously reported effects of amino acids on system y⁺, the exact mechanism is not fully understood [3,6].

When plasma-membrane vesicles were preincubated for 1 h in the presence of 2 mM-putrescine, there was a *trans*-stimulatory effect on L-ornithine transport. Vesicles preloaded with putrescine transported 196 and 148% of the L-ornithine transported by control vesicles for 2 and 10 min respectively. This *trans*-stimulatory effect of putrescine is similar to that reported for cationic amino acids on transport systems y⁺ and Y⁺ in CHO cells [5] and for neutral amino acids in the presence of Na⁺ on system y⁺ in human fibroblasts [3].

Electrostatic interactions of polyamines with anionic components of natural membranes are known to cause more or less specific membrane function changes [17]. In this sense, we have tested the effect of putrescine on the transport of other nutrients avidly consumed by Ehrlich cells, glutamine and glucose [18]. *In vitro*, both substrates modulate the induction of ornithine decarboxylase activity in Ehrlich cells [19], and both are mainly transported by Na⁺-dependent systems [20,21]. Fig. 3 shows that 55 μ M-putrescine stimulates the total transport of 0.5 mM-L-glutamine in membrane vesicles isolated from Ehrlich cells. A reciprocal stimulatory effect of glutamine on putrescine transport has been previously reported for neuroblastoma cells [22]. For brush-border membranes, a specific stimulatory effect of polyamines on D-glucose transport has been reported [17]. However, we could not observe a similar effect on our experimental model in short-time incubations (5, 15 and 60 s) in the presence of 0.5 mM-D-glucose, with and without 55 μ M-putrescine (results not shown).

Fig. 4. Time course of L-ornithine in the presence of several diamines

The transport of 0.5 mM-L-[1-¹⁴C]ornithine by plasma-membrane vesicles was measured in the absence (□) or the presence of histamine (●), 5-hydroxytryptamine (■) or 2,3-diaminopropane (○), each at 55 μ M.

The inhibitory effect of putrescine on L-ornithine transport has been confirmed for other diamines: histamine, 1,3-diaminopropane and 5-hydroxytryptamine showed significant inhibition of L-ornithine transport (Fig. 4). The greatest inhibition was obtained with 55 μ M-5-hydroxytryptamine. This fact correlates well with the maximum decrease in cellular content of L-ornithine observed in Ehrlich cells perfused with 0.5 mM-L-ornithine and 55 μ M-5-hydroxytryptamine [12]. Histamine and 5-hydroxytryptamine, products of histidine decarboxylase and tryptophan decarboxylase reactions, showed, at the pH assayed, a positive charge, similarly to the cationic amino acids.

Some of the reported effects of polyamines on the function of membrane proteins are only due to their cationic properties, and they can be mimicked by bivalent inorganic ions such as Mg^{2+} or Ca^{2+} [17]. We have tested the effect of exogenously added bivalent cations on L-ornithine transport. During the first 2 min of incubation, 55 μ M- Mg^{2+} seemed to enhance L-ornithine uptake slightly; there was no significant difference in the transport of L-ornithine in the absence or in the presence of both 55 μ M- Ca^{2+} and 55 μ M- Mg^{2+} (results not shown). These results suggest that charge is not the only required characteristic to explain the inhibitory effect of diamines.

Evidence is presented for an inhibitory effect of diamines on L-ornithine transport by plasma-membrane vesicles isolated from Ehrlich cells. Polyamine transport is described as an energy- and temperature-dependent and saturable process [23]. Cationic amino acids seem to have a very weak inhibitory effect on polyamine uptake [24]. This fact seems to rule out the possibility of a common transport system for both cationic amino acids and

diamines. Although the actual cause of the inhibitory effect of diamines on L-ornithine transport remains to be elucidated, nevertheless the present paper emphasizes one of the multiple regulatory effects of polyamines at the level of the cytoplasmic membrane [10]. Moreover, the inhibitory effects caused by putrescine and other diamines described as ornithine decarboxylase inhibitors on the uptake of the polyamine precursor ornithine should be considered as an important fact in the understanding of the complex regulation of polyamine metabolism.

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