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A Structure-Relationship Study of the Uptake of Aliphatic Polyamine Compounds by Rat Intestinal Brush-border Membrane Vesicles

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Abstract

The effects of lipophilicity, ion-diffusion potential and membrane surface potential on the uptake of various aliphatic polyamine compounds by rat intestinal brush-border membrane vesicles (BBMV) have been investigated.

A valinomycin-induced potassium-diffusion potential (inside-negative) stimulated the initial uptake of diamine compounds, and good correlation was observed between lipophilicity and the amount of diffusion-potential-dependent transport of the diamines. In contrast, because of their much lower lipophilicity, tri- and tetraamine compounds were not affected by the diffusion potential. Tetracaine, which can make the membrane surface potential more positive, inhibited the transport rate of 1,9-nonanediamine, spermidine and spermine by the BBMV.

These data suggest that the transport mechanism of diamines is similar to that of monoamine compounds in respect to its dependence on ion-diffusion potential and on the membrane surface potential. The extent of the effect of ion-diffusion potential on the rate of transport of the diamines was closely related to the lipophilicity of the diamine. In contrast, only the surface potential contributed to the transport mechanism of lower lipophilic tri- and tetraamine compounds.

Intestinal absorption of polycationic drugs is poor because of their hydrophilicity and the relatively large charge–density within their molecules. The permeation of cationic compounds such as tryptamine, disopyramide and enoxacin across the rat intestinal brush-border membrane has been found to dependent on (interior negative) diffusion potential (Iseki et al 1992; Sugawara et al 1992; Takahashi et al 1993). These reports suggested that the negative diffusion potential inside enterocytes might act as the driving-force of cationic drug absorption from the intestine.

Physiological oligoamines such as spermine and putrescine are essential polyamines for cell growth, differentiation and proliferation (Tabor & Tabor 1984). The cellular content of these compounds are considered to be regulated not only by intracellular synthesis but also by the uptake from extracellular space. There has, however, been little information about the disposition behaviour of these polyamines in the small bowel. Our previous reports showed that the uptakes of spermine and trientine by the intestinal brush-border membrane vesicles (BBMV) were insensitive to changes in the ion-diffusion potential (Kobayashi et al 1993; Tanabe et al 1996), results which are also in agreement with a report of gentamicin uptake by renal BBMV (Lipsky et al 1980). Although it seems, therefore, that there is a difference between the transport characteristics of mono- and oligo-cationic compounds, it is still unclear whether the transport characteristics of the permeation of polycations and mono-cationic compounds are completely different.

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On the basis of this background knowledge we have investigated the uptake characteristics of various polyamine compounds, especially diamine compounds, by rat intestinal BBMV, and demonstrated the relationship between the physicochemical properties of polyamine compounds and the effect of ion-diffusion or membrane potential, or both, on their permeability.

Materials and Methods

Chemicals

Ethylenediamine (diamine 2), 1,3-propanediamine (diamine 3), 1,4-butanediamine dihydrochloride (diamine 4), 1,6-hexanediamine (diamine 6), 1,7-heptanediamine (diamine 7), 1,9-nonanediamine (diamine 9), 1,10-decanediamine (diamine 10) and 3,3′-diaminodipropylamine (triamine 3) were purchased from Wako (Osaka, Japan). 1,8-Octanediamine (diamine-8) was purchased from Nakarai Tesque (Kyoto, Japan). 1,5-Pentanediamine dihydrochloride (diamine 5), *N*-(3-aminopropyl)-1,4-butanediamine trihydrochloride (spermidine), *N,N′*-bis(3-aminopropyl)-1,4-butanediamine tetrahydrochloride (spermine) and valinomycin were obtained from Sigma (St Louis, MO). Fluorescamine was purchased from Fluka (Buchs, Switzerland). Triethylenetetramine dihydrochloride (trientine) was kindly donated by Tsumura & Co. (Tokyo, Japan). All other chemicals were of the highest grade available and were used without further purification.

Determination of the partition coefficient of polyamines in octanol-buffer (pH 7·5)

Each polyamine was dissolved in a buffer of D-mannitol (100 mM), KCl (100 mM), *N*-2-hydroxymethylpiperazine-*N′*-

2-ethanesulphonic acid (HEPES; 20 mM) and tris(hydroxymethyl)aminomethane (Tris; 20 mM) adjusted to pH 7·5. *n*-Octanol (buffer-saturated; 5 mL) was added to diamine solution (0·5 mL) in a glass tube and shaken for 30 min. The mixture was centrifuged at 1000 *g* for 10 min, and the organic layer (4 mL) was transferred to a different tube. Polyamine-free buffer (0·5 mL) was added and the mixture was shaken for 30 min and then centrifuged at 1000 *g* for 10 min. The amount of polyamine compound in the buffer layer was determined by HPLC.

Isolation of brush-border membrane vesicles (BBMV) from the small intestine of the rat and polyamine uptake experiments

Experiments were performed on adult male Wistar rats, 200–250 g. The entire small intestine was excised under anaesthesia (sodium pentobarbitone; 50 mg kg^{-1}, i.p.). Brush-border membrane vesicles were isolated by use of the calcium precipitation method of Kessler et al (1978) as described previously (Iseki et al 1991).

The uptake of polyamine compounds into BBMV was measured by a rapid filtration technique as described previously (Iseki et al 1991). In the routine assay, membrane suspension (20 μL; 10–15 mg protein mL^{-1}) were added to incubation medium (for composition see figure legends; 100 μL) kept at 37°C. At selected time intervals, uptake was stopped by diluting the incubation medium with ice-cold HEPES-Tris buffer (pH 7·5; 10 mM; 3 mL) containing KCl (150 mM). The mixture was immediately filtered through a Millipore filter (HAWP), 0·45 μm, 2·5 cm diameter). The filter was washed once with the same ice-cold buffer. The substrate trapped on the filter was extracted with phosphate buffer (pH 7·5; 5 mM; 500 μL) containing KCl (500 mM).

In this study, uptake at 4°C for 5 s was considered as binding to the membrane surface, and the transport rate of diamines was estimated by subtracting the binding value from total uptake at 37°C for 30 s.

Analytical methods

Before analysis polyamine compounds were pre-labelled with fluorescamine. Phosphate buffer (pH 9·5, 100 mM; 200 μL) then fluorescamine solution in acetonitrile (1 mM; 50 μL) were added to the sample (200 μL), which was then mixed vigorously for 30 s by use of a vortex mixer. When the reaction was over and excess fluorescamine had completely decomposed (10 min), α-naphthylamine (50 μM; 50 μL; for diamines 2, 3, 4, 5, 6, 8 and triamine 3, spermidine, spermine, trientine) or naphthoresorcinol (500 μM; 50 μL; for diamines 7, 9, 10) were added to the reaction mixture as internal standard.

Analysis was performed by HPLC (Hitachi L-6000, Hitachi, Tokyo); the chromatograph was equipped with a Rheodyne 7125 (Cotati, CA) sample injector and multi-wavelength fluorimetric detector (820-FP, Jasco, Tokyo) which was used at an excitation wavelength of 380 nm and an emission wavelength of 485 nm. Fluorescamine-labelled diamines 2–10 were separated on a 250 × 4 mm i.d., particle size 5 μm, ODS column (Hitachi Gel #3053; Hitachi, Tokyo, Japan). The column was maintained at 40°C and the mobile phase was 30% acetonitrile, 2 mM sodium dodecylsulphonate and 6·7 mM acetic acid (pH adjusted to 6·0 with NaOH). The flow rate was 0·7 mL min^{-1} and the pressure approximately 70 kg cm^{-2}. The other polyamines were determined according to a method reported previously (Miyazaki et al 1990) with minor modifications (Tanabe et al 1996) using a nitrile column (Nucleosil 5-CN, 250 × 4 mm i.d., particle size 5 μm; Macherey-Nagel, Duren, Germany). The column was maintained at 40°C and the mobile phase was 27% of acetonitrile, 100 mM ammonium chloride, 35 mM benzenesulphonate and 6·7 mM acetic acid (pH adjusted to 6·0 with NaOH). All the polyamine compounds were eluted within 20 min, and the limits of detection were at least 10 pmol in the extraction sample from the membrane filter.

Protein concentration was determined by the method of Lowry et al (1951) with bovine serum albumin as the standard. The experiments presented in this paper were repeated using at least three different preparations, and were always performed in triplicate. Statistical analysis was performed by use of the unpaired Student's *t*-test and $P < 0.05$ was considered as indicative of significance. In the experiment to determine correlation between diffusion-potential-induced transport rate and partition coefficient it was not appropriate to analyse the data by this statistical method because the datum for diamine-10 was apart from other data points. These data were, therefore, examined by non-parametric analysis and Spearman's rank correlation was calculated by Abacus StatView (v. 4·5, Berkeley, CA) on a Macintosh computer.

Results

Partition coefficient between octanol and buffer (pH 7·5) and transport rate of polyamine compounds by BBMV

As illustrated in Table 1, the octanol–buffer partition coefficients (P_{oct}) of diamines larger than diamine 5 increased as the N–N distance increased. In contrast, the P_{oct} of the short chain-length diamines (diamines 2 to 5) showed the opposite behaviour. The P_{oct} of the tri- and tetraamine compounds were much lower than those of the diamines. That of spermine, in particular, could not be determined, because it was $< 10^{-6}$.

Because polyamine compounds were reported to bind to the membrane surface as a result of their cationic charges (Schuber 1989), the uptake values obtained from the BBMV experiment included binding to the membrane and the transport into the

Table 1. *n*-Octanol-buffer (pH 7·5) partition coefficients of different polyamine compounds.

Compound	Partition coefficient ($\times 10^{-4}$)
Diamines	
Ethylenediamine	6·22 ± 0·22
1,3-Propanediamine	2·83 ± 0·31
1,4-Butanediamine	3·81 ± 0·53
1,5-Pentanediamine	2·77 ± 0·58
1,6-Hexanediamine	2·87 ± 0·39
1,7-Heptanediamine	3·30 ± 0·32
1,8-Octanediamine	4·85 ± 0·41
1,9-Nonanediamine	6·55 ± 0·85
1,10-Decanediamine	15·11 ± 0·42
Triamines	
3,3′-Diaminodipropylamine	0·11 ± 0·028
Spermidine	0·068 ± 0·013
Tetraamines	
Trientine	0·074 ± 0·011
Spermine	< 0·01

Each value represents the mean ± s.e. of 5–9 measurements.

vesicles. In the current study the initial transport rate of diamine 9 into the BBMV was linear for approximately 30 s ($r = 0.973$). The y-intercept of this line, i.e. the binding to the membrane, was almost the same as the uptake value at 4°C for 5 s. The initial uptake behaviour of the tri- and tetraamine compounds spermidine and trientine was the same as that of diamine 9 (data not shown). The uptake of polyamine compounds at 4°C for 5 s was, therefore, considered as 'binding' to the membrane surface, and the transport rate was estimated by subtracting the 'binding' value from the total uptake at 37°C for 30 s.

Fig. 1 shows there was good correlation between the transport rate of polyamine compounds and the P_{oct}. The transport rate of tri- and tetraamine compounds was, on the other hand, almost the same as that of diamine 7, even though the lipophilicity of tri- and tetraamine compounds was much lower than for diamines.

Effect of potassium-diffusion potential on the uptake of diamines

As shown in Table 2, except for diamine 6 the uptake of diamines was significantly stimulated by a potassium diffusion potential (inside negative). The increase in the rate of transport caused by the potassium diffusion potential, furthermore, correlated strongly with the compound lipophilicity (Fig. 2), although the overshoot phenomenon was not observed in any diamine compound.

The inhibitory effect of tetracaine on the transport rate of polyamine compounds

Table 3 shows the effect of tetracaine on the transport rates of diamine 9, spermidine and spermine into BBMV. The transport rates of all the compounds were markedly inhibited by tetracaine, and the inhibitory effect was concentration-dependent. Our previous study demonstrated that tetracaine inhibits the uptake of monoamine compounds, such as tryptamine, into BBMV, and that this was caused by the decreasing effect of tetracaine on the membrane surface potential (Sugawara et al 1995). These data obtained from polyamine experiments were in approximate agreement with the tryptamine uptake behaviour across the brush-border membrane. It seems that membrane surface potentials play a common role in the transport of mono- and poly-cationic compounds.

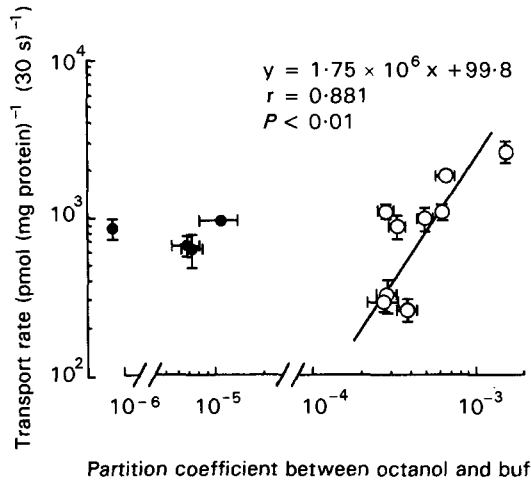

FIG. 1. Correlation between the initial transport rate of polyamine compounds by rat intestinal BBMV and the partition coefficient. Membrane vesicles (20 μL) were suspended in 100 mM KCl, 100 mM D-mannitol and 20 mM HEPES-Tris buffer (pH 7·5). Uptake studies were performed by adding an incubation medium (100 μL) containing 100 mM KCl, 100 mM D-mannitol, 20 mM HEPES-Tris buffer (pH 7·5) and 1·2 mM polyamine compounds. ○, Diamines; ●, tri- and tetraamines. Each point represents the mean ± s.e. of 5–12 measurements.

Table 2. Effect of a valinomycin-induced potassium diffusion potential (inside negative) on the initial uptake (30 s) of diamines by rat intestinal BBMV.

	Uptake at 30 s (pmol (mg protein)$^{-1}$)		
	Control	Inside negative	n
Ethylenediamine	428·6 ± 49·8	821·0 ± 87·4*	6
1,3-Propanediamine	880·1 ± 55·0	1019·4 ± 35·2*	10
1,4-Butanediamine	99·3 ± 38·2	261·3 ± 47·5*	9
1,5-Pentanediamine	193·0 ± 38·1	289·3 ± 29·9*	10
1,6-Hexanediamine	425·8 ± 43·8	523·4 ± 65·7	7
1,7-Heptanediamine	753·6 ± 51·5	951·4 ± 83·3*	12
1,8-Octanediamine	886·3 ± 78·9	1202·1 ± 73·8*	6
1,9-Nonanediamine	1709·6 ± 98·6	2348·5 ± 225·4*	8
1,10-Decanediamine	4123·7 ± 238·8	5922·3 ± 203·2**	6

Membrane vesicles (20 μL) were suspended in a solution of potassium gluconate (100 mM), D-mannitol (100 mM) and HEPES-Tris buffer (pH 7·5, 20 mM). Uptake studies were performed by adding an incubation medium (100 μL) containing D-mannitol (100 mM), HEPES-Tris buffer (pH 7·5, 20 mM) and diamine (1·2 mM) and either sodium gluconate (inside negative; 100 mM) or potassium gluconate (control). Each value represents the mean ± s.e. of 6–12 measurements. *$P < 0.05$, **$P < 0.01$, compared with control.

Table 3. Inhibitory effect of tetracaine on the transport rate of 1,9-nonanediamine, spermidine and spermine (1 mM) by rat intestinal BBMV.

	Polyamine transport rate (% of control)		
Tetracaine	1,9-Nonanediamine	Spermidine	Spermine
0 mM (control)	100 ± 9·2	100 ± 5·4	100 ± 17·0
2 mM	33·6 ± 5·7	33·0 ± 6·2	0·1 ± 14·2
4 mM	10·4 ± 1·3	0 ± 4·5	3·8 ± 11·7
8 mM	3·4 ± 1·9	0 ± 4·1	7·0 ± 10·8

Each value represents the mean ± s.e. of 6–14 measurements.

Discussion

The impact of structural features of the polyamine compounds on their uptake by rat intestinal BBMV was investigated. The transport rates of tri- and tetraamine compounds were found to be clearly related to the lipophilicity (P_{oct}). It is well known that polyamine compounds bind to acidic molecules, depending on the number of cationic amine groups in their molecules (Yung & Green 1986). We have previously reported that the binding of spermine and spermidine to phosphatidylserine, which is localized at the inner leaflet of the biomembrane, is related to the force of their transmembrane transport (Iseki et al 1991; Kobayashi et al 1992). It is, therefore believed that the diversity of quantitative ratios of net charge per molecule among the polyamines is the reason for the deviation from the lipophilicity–transport correlation.

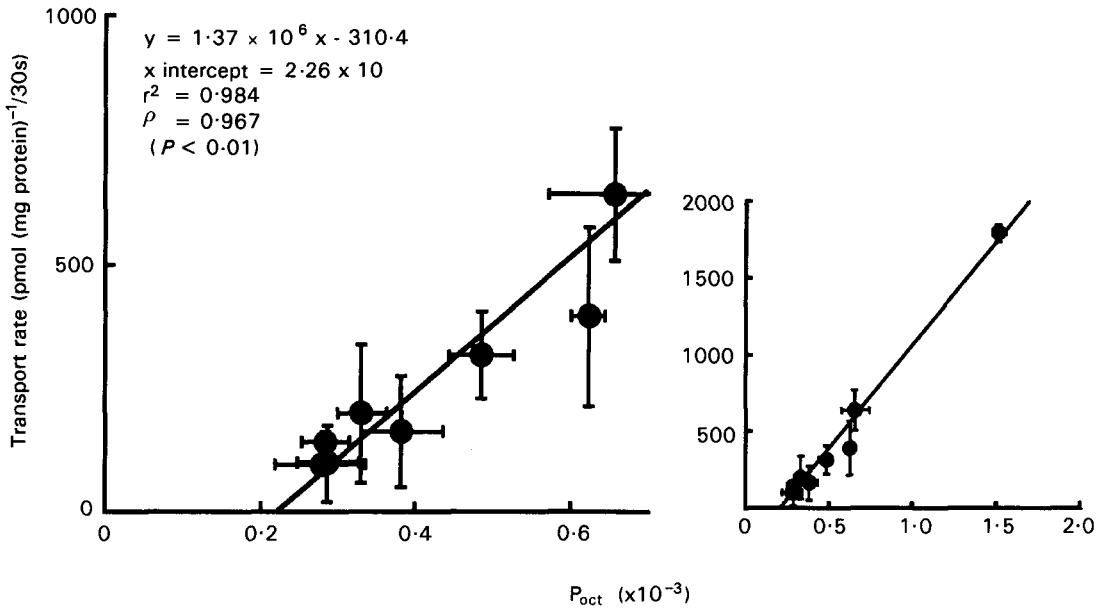

FIG. 2. Correlation between the diffusion-potential-induced transport rate (30 s) of diamines by rat intestinal BBMV and partition coefficient. Each point represents the mean ± s.e. of 6–12 measurements. ρ, Spearman's rank correlation.

Our previous reports showed that the initial uptake of monocationic compounds (Iseki et al 1992; Sugawara et al 1992; Takahashi et al 1993) was strongly stimulated by potassium diffusion potential (inside negative). The initial transport of diamines, particularly the highly lipophilic diamines, was also stimulated by a diffusion potential. In this study, a good correlation was observed between lipophilicity and the net transport induced by the diffusion potential (Fig. 2), although the stimulation of diamine transport was less than that of tryptamine owing to a lack of the overshoot phenomenon. These data suggest that an inside-negative ion-diffusion potential can enhance the transport of cationic compounds, and that the degree of stimulation is related to the lipophilicity. Because tryptamine is more lipophilic than diamines ($P_{oct} \approx 10^{-1}$), it seems to be strongly stimulated by the inside-negative ion-diffusion potential. This speculation is also supported by the observation that the uptake of the compound whose P_{oct} is lower than $2{\cdot}26 \times 10^{-4}$ is not affected by the potassium diffusion potential alone. We previously reported that the uptake of spermidine and trientine ($P_{oct} < 10^{-5}$) was not stimulated by the presence of an inside-negative potential (Kobayashi et al 1993; Tanabe et al 1996). Some lipophilicity is, therefore, thought to be necessary for the diffusion potential-sensitive permeation of cationic compounds.

As shown in Table 3, the initial uptake of diamine 9, spermidine and spermine were, on the other hand, found to be inhibited by tetracaine, which can modify the outer-membrane surface potential of the BBMV (Sugawara et al 1995). Because tetracaine can make the membrane surface potential more positive, it is thought that diamine transport is also affected by the negative charge of the outer membrane surface. It has been confirmed that these polycations interact with both inner- and outer-membrane surfaces (Kobayashi et al 1993).

In conclusion, the mechanisms of transport of mono- and diamine compounds by rat intestinal BBMV were almost identical in terms of their dependence on ion-diffusion and membrane-surface potentials. The transport of the tri- and tetraamine compounds used in this study was, on the other hand, because of their low lipophilicity, not affected by the ion-diffusion potential.

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