J. Pharm. Pharmacol. 1999, 51: 279–284
Received October 12, 1998
Accepted November 5, 1998

The Presence of an Na^+/Spermine Antiporter in the Rat Renal Brush-border Membrane

MICHIYA KOBAYASHI, HIROKO FUJISAKI, MITSURU SUGAWARA, KEN ISEKI AND KATSUMI MIYAZAKI

Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Sapporo 060-8648, Japan

Abstract

This study was aimed at determining the driving force for spermine transport in rat renal proximal tubular brush-border membrane.

The uptake of spermine and trientine, a spermine-like drug used for treating Wilson's disease, into rat renal brush-border membrane vesicles was significantly stimulated by an outwardly directed Na^+ gradient. The Na^+-dependent uptake was temperature dependent and saturable. A kinetic analysis of the initial uptake of spermine with an Na^+ gradient gave a K_m value of 1·44 μM and a V_{max} value of 6·31 pmol (mg protein)$^{-1}$/30 s. The Na^+-dependent uptake of [^{3}H]spermine was inhibited by spermine, trientine and tetraethylenepentamine. Substrates of the H^+/organic cation transporter (cimetidine and tetraethylammonium), physiological polyamines (putrescine and spermidine) with 2 or 3 amino groups and aminoglycosides (amikacin and tobramicin) with 4 or 5 cationic amines did not affect the uptake of spermine in the presence of an outwardly directed Na^+ gradient.

These results suggest that the renal tubular secretion of spermine is mediated by an Na^+/spermine antiport system which is specific for a straight-chain polyamine compound with more than 4 amino groups.

Spermine, a polyamine compound, plays an important role in the proliferation and growth of cells (Tabor & Tabor 1976; Pegg & McCann 1982). It is well known that physiological polyamines are taken up by various mammalian cells via specific carrier systems (Seiler & Dezeure 1990; Seiler et al 1996). The energy of many of these systems is provided by an inwardly directed Na^+ gradient (Gawel-Thompson & Greene 1988; Kameji et al 1989; Van den Bosch et al 1990; Khan et al 1991). We have also reported on the presence of Na^+/spermine and Na^+/putrescine co-transporters in the rat intestinal brush-border (Iseki et al 1991) and basolateral membranes (Kobayashi et al 1995), respectively.

In our recent study (Kobayashi et al 1997), we found a specific transporter that recognizes spermine and trientine, a spermine-like drug used for treating Wilson's disease (Figure 1), in the rat renal brush-border membrane. In-vivo clearance studies (Kobayashi et al 1997) indicated that this transporter contributes to the active secretion of spermine and trientine into the luminal side of the renal proximal tubule. However, this system was not an Na^+/spermine symporter, and moreover, the H^+/organic cation antiporter, which secretes many kinds of organic cations such as cimetidine (Takano et al 1985) and tetraethylammonium (Takano et al 1984) into the renal tubule, does not recognize these polyamines despite the fact that spermine and trientine are polycationic compounds.

In the present study, we have investigated the uptake behaviour of spermine and trientine in the rat renal proximal brush-border membrane in order to uncover the driving force and the characteristics of this transport system.

Correspondence: Katsumi Miyazaki, Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060-8648, Japan. E-mail: katsumim@med.hokudai.ac.jp

Materials and Methods

Chemicals

[^{3}H] Spermine tetrahydrochloride (1·48 TBq mmol^{-1}) was purchased from American Radio-

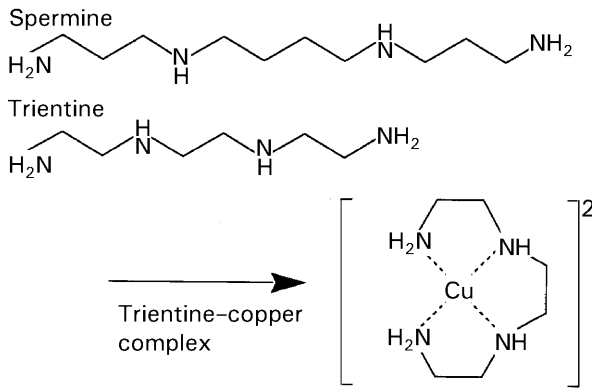

Figure 1. Chemical structures of spermine, trientine, trientine–copper complex.

labeled Chemicals (St Louis, MO). Trientine dihydrochloride (trientine) was kindly donated by Tsumura (Tokyo, Japan). Methylglyoxal bis(guanylhydrazone) (MGBG) was purchased from Aldrich Chemical (Milwaukee, WI). Amikacin, Tobramicin, and N^1-methylnicotinamide (NMN) were purchased from Sigma (St Louis, MO). Other chemicals were of the highest grade available and were used without further purification.

Isolation of brush-border membrane vesicles from the rat kidney cortex

Renal brush-border membrane vesicles were prepared from the kidney cortex of white Wistar rats (200–250 g, maintained on a normal diet with free access to food and water) by the Mg^{2+}/EGTA precipitation method according to Biber et al (1981) with several modifications as described in our previous report (Kobayashi et al 1997). Cortex slices were homogenized using a Waring blender (Nihon Seiki, Japan) at 16 500 rev min^{-1} for 4 min in an appropriate volume of homogenizing buffer composed of 300 mM D-mannitol, 12 mM tris(hydroxymethyl) aminoethane (Tris)/HCl (pH 7.1) and 5 mM EGTA. The homogenate was diluted with distilled water (1 : 1) and $MgCl_2$ was added to a final concentration of 10 mM. After 15 min, the mixture was centrifuged at 19 000 *g* for 15 min. The supernatant was then centrifuged at 27 000 *g* for 30 min. An appropriate volume of the homogenizing buffer was diluted with distilled water (1 : 1) and used to resuspend the resulting pellets. The pellet suspension was homogenized in a Douce-type homogenizer (10 strokes). Magnesium chloride was added to a final concentration of 10 mM and the first two steps of centrifugation repeated. The resulting pellets (brush-border membrane vesicles) were washed and resuspended in a pre-loading buffer containing 100 mM D-mannitol and 100 mM NaCl with 20 mM *N*-2-hydroxymethylpiperazine-*N*′-2-ethanesulphonic acid (HEPES)/Tris for pH 7·0 and 7·5, or with 20 mM 2-morpholinoethanesulphonic acid (MES) for pH 6·5. Membrane purification was routinely checked by measuring alkaline phosphatase, which is increased more than 10-fold in the final membrane suspension compared with the concentration in the homogenated kidney cortex.

Uptake study

Uptake studies were performed at 37°C using a rapid filtration technique reported previously (Kobayashi et al 1997). The reaction was started by mixing the membrane suspension (20 μL) with an incubation medium (100 μL) containing substrates. At the stated time intervals the reaction was terminated by dilution with ice-cold stop buffer (150 mM KCl, 10 mM HEPES/Tris, pH 7·5 or 7·0 or MES/Tris, pH 6·5; 4 mL), and the solution was then filtered through a Millipore filter (HAWP, 0·45 μm, 2·5 cm diameter). The filter was washed once with 4 mL of the ice-cold stop buffer. All experiments represented in this paper were repeated on three different preparations and were always performed in triplicate.

Analytical methods

The detection of trientine in the brush-border membrane vesicles trapped on the filter was performed by HPLC (Hitachi L-6000; Hitachi, Tokyo) with fluorimetric detection (820-FP, Jasco, Tokyo) as described previously (Kobayashi et al 1997). The [^{3}H]-labelled spermine was measured by liquid scintillation counting. Protein concentration was determined by the method of Lowry et al (1951) with bovine serum albumin as the standard. Statistical analysis was performed using Student's unpaired *t*-test, and $P < 0{\cdot}05$ was considered significant.

Results

Effect of an outwardly directed Na^+ gradient on the uptake of spermine by brush-border membrane vesicles

Table 1 illustrates the effect of an outwardly directed Na^+ gradient on the uptake of spermine by renal brush-border membrane vesicles. The initial uptake of spermine was slightly, but significantly, stimulated in the presence of an Na^+ gradient. Previously, we have found that spermine binds strongly to the brush-border membrane at this pH value (Kobayashi et al 1992, 1995). We have also

reported that the amount of spermine bound to the plasma membrane decreases as the medium pH decreases (Kobayashi et al 1992). These findings suggest that the carrier-mediated uptake of spermine is masked by the large amount of binding to the membrane. Therefore, to decrease the role of binding in the uptake process, we investigated the uptake of spermine at pH 7·0 and 6·5. As shown in Figure 2, the initial uptake of spermine was significantly stimulated at both levels of pH. The Na^+-dependent uptake (outward Na^+ gradient uptake minus non-ion gradient uptake) for each pH condition at 30 seconds was $6{\cdot}76 \pm 0{\cdot}86$ pmol (mg protein)$^{-1}$ (pH 6·5), $6{\cdot}94 \pm 0{\cdot}78$ (pH 7·0) and $7{\cdot}37 \pm 1{\cdot}07$ (pH7·5). These data were not statistically different; therefore, it can be considered that the medium pH does not affect the spermine transporter activity.

These data suggest that the energy of the transporter for the uptake of spermine is an outwardly directed Na^+ gradient. It is thought, however, that an inwardly directed K^+ gradient might also play a role in the uptake process. To clarify this point, we examined the uptake using brush-border membrane vesicles prepared by an intra- or an extra-vesicular medium containing LiCl instead of NaCl or KCl. As shown in Table 2, an outwardly directed Na^+ gradient (K^+_{out}/Na^+_{in} and Li^+_{out}/Na^+_{in}) stimulated the uptake of spermine, and stimulation of the uptake of spermine (K^+_{out}/Na^+_{in}) decreased when the intra-vesicular Na^+ ion was replaced by Li^+ (K^+_{out}/Li^+_{in}). On the contrary, when the extra-vesicular K^+ ion was replaced by Li^+ (Li^+_{out}/Na^+_{in}), stimulation of the uptake of spermine was not changed.

Table 1. Effect of an outward Na^+ gradient on the uptake of spermine by rat renal brush-border membrane vesicles at pH 7·5.

Incubation time	Spermine uptake (pmol (mg protein)$^{-1}$)	
	Na^+_{out}/Na^+_{in}	K^+_{out}/Na^+_{in}
30 s	$15{\cdot}78 \pm 1{\cdot}23$	$20{\cdot}89 \pm 1{\cdot}92$
1 min	$17{\cdot}11 \pm 2{\cdot}67$	$26{\cdot}93 \pm 2{\cdot}52$*
30 min	$31{\cdot}77 \pm 0{\cdot}99$	$33{\cdot}41 \pm 2{\cdot}39$

Membrane vesicles (20 μL) were suspended in 100 mM NaCl, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·5). Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·5), 2·4 μM spermine (final concentration was 2 μM) and either 100 mM NaCl or KCl as shown. Results are presented as mean ± s.e.m. of 3 preparations. *$P < 0{\cdot}05$ compared with Na^+_{out}/Na^+_{in}.

It is well known that the activity of carrier proteins is temperature dependent. As illustrated in the left of Figure 2, the Na^+-dependent uptake of spermine disappeared completely when the experiment was carried out on ice, strongly suggesting the presence of an Na^+/spermine antiporter in the rat renal brush-border membrane.

Kinetic parameters of the Na^+/spermine antiporter

The Na^+-dependent uptake of spermine (after subtracting the uptake value at K^+_{out}/K^+_{in} from the uptake at K^+_{out}/Na^+_{in}) was saturable at a con-

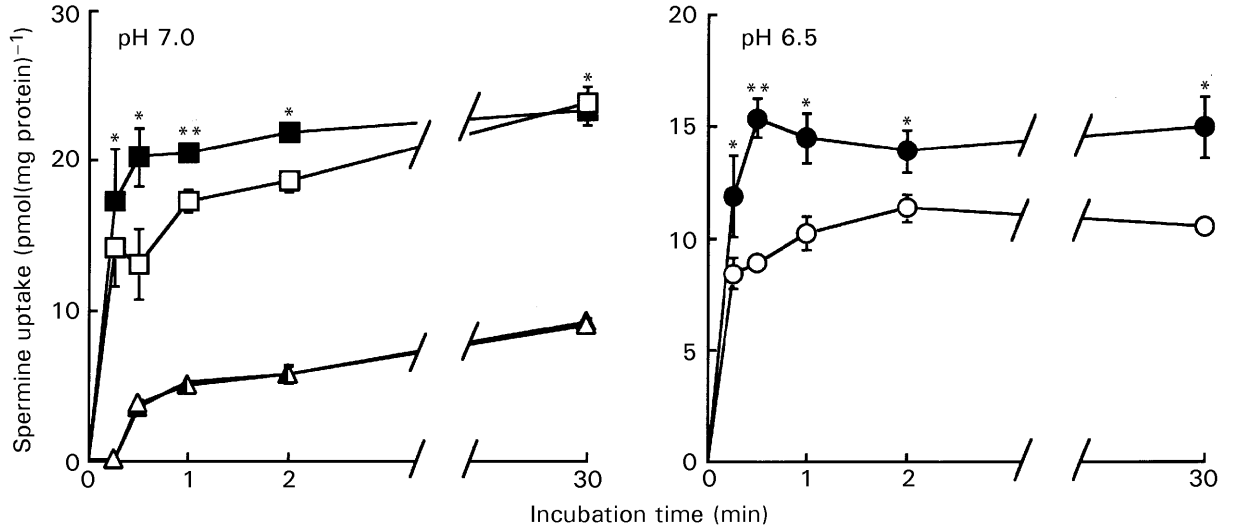

Figure 2. Effect of an outward Na^+ gradient on the uptake of spermine by rat renal brush-border membrane vesicles at pH 7·0 (left) and pH 6·5 (right). Membrane vesicles (20 μL) were suspended in 100 mM NaCl, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·0) or MES/Tris (pH 6·5). Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0) or MES/Tris (pH 6·5), 2·4 μM spermine (final concentration was 2 μM) and 100 mM NaCl (open symbols) or KCl (closed symbols). Medium temperature was 37°C (squares and circles) or 4°C (triangles). Each point represents the mean ± s.e.m. of 3 preparations. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with Na^+_{out}/Na^+_{in}.

Table 2. Effect of an inorganic ion gradient on the uptake of spermine by rat renal brush-border membrane vesicles.

Time (min)	Uptake (pmol (mg protein)$^{-1}$)[a]			
	Na^+_{out}/Na^+_{in}	K^+_{out}/Na^+_{in}	Li^+_{out}/Na^+_{in}	K^+_{out}/Li^+_{in}
0·5	13·92 ± 1·32	21·29 ± 0·63**	20·12 ± 1·06*	16·93 ± 1·50$^+$
1	16·59 ± 0·92	21·76 ± 0·46**	20·50 ± 1·18*	18·13 ± 1·40$^+$
2	18·11 ± 0·46	22·82 ± 0·65**	22·58 ± 1·76*	21·20 ± 1·62
30	22·68 ± 1·41	24·20 ± 1·20	23·66 ± 0·80	23·81 ± 1·37

Membrane vesicles (20 μL) were suspended in 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0) and 100 mM NaCl or LiCl. [a]Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0), 2·4 μM spermine (final concentration was 2 μM) and 100 mM NaCl, KCl or LiCl. Each value represents the mean ± s.e.m. of 3 preparations. *$P < 0·05$, **$P < 0·01$ compared with Na^+_{out}/Na^+_{in}, $^+P < 0·05$ compared with K^+_{out}/Na^+_{in}.

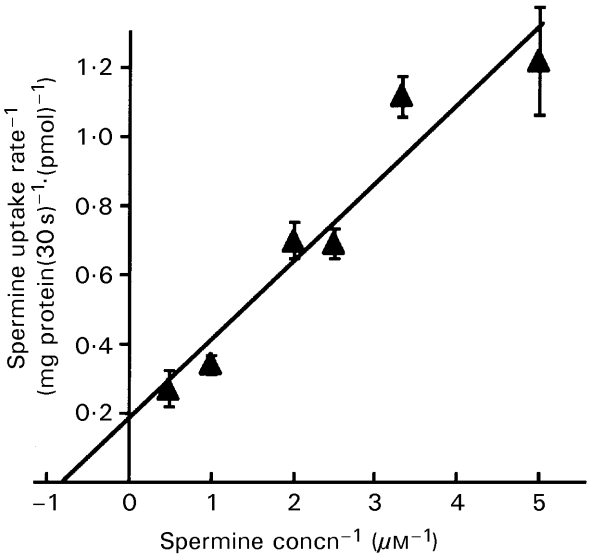

Figure 3. Lineweaver-Burk plot of the Na^+-dependent initial uptake (30 s) of spermine by rat renal brush-border membrane vesicles. Membrane vesicles (20 μL) were suspended in 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0) and 100 mM KCl or NaCl. Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0), 0·24–24 μM spermine (final concentration was 0·2–20 μM) and 100 mM KCl. The Na^+-dependent uptake was calculated by subtracting the uptake value at K^+_{out}/K^+_{in} from the uptake at K^+_{out}/Na^+_{in}. The regression line was calculated by the standard least-squares program using a Macintosh computer. The intercepts of the line with the x- and y-axes are: $-K_m^{-1}$ ($K_m = 1·44$ μM) and V_{max}^{-1} ($V_{max} = 6·31$ pmol (mg protein)$^{-1}$ (30 sec)$^{-1}$). Each point represents the mean ± s.e.m. of 3 preparations.

centration of 0·2–20 μM. The kinetic parameters obtained from a Lineweaver-Burk plot (Figure 3) were as follows: $K_m = 1·44$ μM and $V_{max} = 6·31$ pmol (mg protein)$^{-1}$ (30 s)$^{-1}$. The K_m value of this transporter is in good agreement with the values reported using cell lines derived from various organs (Seiler & Dezeure 1990).

Characteristics of the Na^+/spermine antiporter

In our previous study (Kobayashi et al 1997), trientine, a therapeutic drug for Wilson's disease, was found to be recognized by the same transport system as that for spermine in the rat renal brush-border membrane vesicles, while trientine-copper chelate complex was not. Therefore, it would be interesting to test whether trientine behaves similarly in the case of the Na^+/spermine antiporter. As shown in Table 3, the uptake of trientine was stimulated by an outwardly directed Na^+ gradient, whereas there was no effect of the Na^+ gradient on the uptake of trientine-copper complex. To clarify the substrate specificity of this transporter, we examined the inhibitory effect of various amine compounds on the uptake of spermine. As shown in Table 4, the passive diffusion of spermine (the amount transported in the absence of an Na^+ gradient) was not inhibited by any of the amine compounds. On the contrary, the Na^+-dependent transport of spermine was significantly inhibited by spermine, trientine and tetraethylenepentamine, which have 4 or 5 amino groups. Meanwhile, tetraethylammonium, cimetidine and N^1-methylnicotinamide (NMN), well-known substrates for the H^+/organic cation antiporter; MGBG, a substrate for the Na^+/putrescine co-transporter in the rat

Table 3. Effect of an outward Na^+ gradient on the uptake of trientine and trientine-Cu complex by rat renal brush-border membrane vesicles.

	Time	Uptake (pmol (mg protein)$^{-1}$)[a]	
		Na^+_{out}/Na^+_{in}	K^+_{out}/Na^+_{in}
Trientine	30 s	171·0 ± 21·8	279·2 ± 13·8**
	60 s	340·0 ± 17·7	423·7 ± 23·6*
Trientine+Cu^{2+}	30 s	147·7 ± 19·8	158·3 ± 11·0
	60 s	239·4 ± 28·8	224·0 ± 58·6

Membrane vesicles (20 μL) were suspended in 100 mM NaCl, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·0). [a]Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0), 1·2 mM trientine (final concentration was 1 mM), 100 mM NaCl or KCl and with or without 1·2 mM $CuSO_4$. Each value represents the mean ± s.e.m. of 3 preparations. *$P < 0·05$, **$P < 0·01$ compared with Na^+_{out}/Na^+_{in}.

Table 4. Inhibitory effect of various polyamines on the Na^+-independent and Na^+-dependent initial uptake of spermine (2 μM) by rat renal brush-border membrane vesicles.

Inhibitors (100 μM)	Uptake (pmol (mg protein)$^{-1}$)[a]	
	Na^+_{out}/Na^+_{in}	K^+_{out}/Na^+_{in}
No additive	13·43 ± 0·21	17·83 ± 0·45
Putrescine	12·72 ± 0·89	18·02 ± 0·21
Spermidine	13·65 ± 0·65	17·93 ± 0·31
Spermine	12·97 ± 0·77	12·97 ± 0·32**
Trientine	13·28 ± 0·81	12·05 ± 0·80**
TEPA	13·53 ± 0·30	15·14 ± 0·21*
TEPA (500 μM)	12·15 ± 0·15	12·54 ± 0·24**
Amikacin	14·06 ± 0·22	17·16 ± 0·64
Tobramicin	13·44 ± 0·35	17·58 ± 0·14
TEA	13·29 ± 0·44	17·30 ± 0·49
Cimetidine	13·43 ± 0·10	17·56 ± 0·44
NMN	14·20 ± 0·36	17·53 ± 0·22
MGBG	13·23 ± 0·27	17·53 ± 0·32

Membrane vesicles (20 μL) were suspended in 100 mM NaCl, 100 mM D-mannitol and 20 mM HEPES/Tris (pH 7·0). [a]Uptake study was performed by adding an incubation medium (100 μL) containing 100 mM D-mannitol, 20 mM HEPES/Tris (pH 7·0), 2·4 μM spermine (final concentration was 2 μM), 100 mM NaCl or KCl and 120 μM of various amine compounds. Each value represents the mean ± s.e.m. of 3 preparations. *$P < 0·05$, **$P < 0·01$ compared with no additive. TEPA, tetraethylenepentamine; TEA, tetraethylammonium; NMN, N^1-methylnicotinamide; MGBG, methylglyoxal bis (guanylhydrazone).

intestinal basolateral membrane (Kobayashi et al 1995); and amikacin and tobramicin, aminoglycoside antibiotics with 4 or 5 amino groups did not inhibit the Na^+-dependent transport of spermine. Furthermore, the transport of spermine was not inhibited by putrescine and spermidine, which are physiological polyamines having 2 and 3 amino groups in their molecules, respectively. These data clearly suggest that the Na^+/spermine antiporter recognizes the straight-chain polyamine compounds that have more than 4 amino groups.

Discussion

In the present study, we found that the Na^+/spermine antiporter is responsible for the active secretion of spermine and trientine into the luminal side of the rat renal proximal tubule. The inhibition of [^{3}H]spermine uptake induced by spermine, trientine, TEPA, MGBG, substrates for the H^+/organic cation antiporter, aminoglycosides, and physiological polyamines other than spermine indicates that the Na^+/spermine antiport system is engaged actively in the transport of straight-chain polyamine compounds with more than 4 amino groups. Furthermore, a chelate form of trientine with copper abolished the ability of the Na^+/spermine antiporter to recognize trientine. The change that occurred on the four cationic amines of trientine after chelating with the copper ion might be the reason for the lack of recognition by the antiporter. Acetylpolyamines, metabolites of polyamines, were abundant in urine, especially in tumour-bearing organisms (Abdel-Monem et al 1982; Takenoshita et al 1984; Kurihara et al 1993). It has not been ascertained how these metabolites are excreted from cells (Seiler et al 1996). In this study, it was not clarified whether the Na^+/spermine antiporter recognizes acetylspermine. The cationic charge of spermine is reduced by *N*-acetylation because acetylated nitrogen is not protonated at physiological pH. Therefore, it was thought that this transporter does not recognize acetylated spermine.

Thus far, only inorganic ions such as H^+ (Murer et al 1976) and Ca^{2+} (Rowe et al 1991) have been reported as substrates for Na^+ antiporters. There have only been a few reports describing polyamine transporters that are responsible for the efflux of polyamines to the extra-cellular space, an ornithine/putrescine antiport system in *Escherichia coli* (Kashiwagi et al 1997), and putrescine (Fukumoto & Byus 1997) and spermidine (Sha et al 1996) efflux systems in *Xenopus* oocytes. For mammalian cells, Fukumoto & Byus (1996) reported the presence of a putrescine and spermidine export system in the human erythrocyte. In all of the above studies, however, the driving force and the characteristics of the export process of polyamines from cells have not been identified. To our knowledge, the Na^+/spermine antiporter in this study is an unprecedented system for the transport of polyamines in mammalian cells.

The polyamines, spermidine and spermine, and their precursor, putrescine, are ubiquitous and found in all cells of higher eukaryotes (Pegg & McCann 1982). The exact function of polyamines at the molecular level is still obscure, but their concentrations appear to be highly regulated. Recent studies (Pegg 1986; Seiler 1987, 1990) have shown that the regulation of intracellular polyamine concentration is mainly based on biosynthesis and transport (uptake and release). More recently, we have reported on the presence of an Na^+/putrescine co-transport system in the basolateral membrane of rat small intestinal epithelial cells (Kobayashi et al 1995). This co-transporter contributes to the uptake of putrescine, but not spermidine and spermine, from the serosal side into the epithelial cells. On the other hand, in the renal brush-border membrane, only spermine was found to be recognized by the Na^+/spermine antiporter and was actively excreted into the lumen. It is well known that the binding of spermine to endogenous

acidic compounds such as phosphatidylserine (Yung & Green 1986) and nucleic acid (Feuerstein et al 1990) is the strongest among the physiological polyamines. Therefore, the interaction of spermine with the bio-membrane and DNA is more active than that of spermidine and putrescine. It is considered that the Na^+/spermine antiporter, which works to decrease the concentration of the finally synthesized and the most active polyamine, spermine, in cells, might play an important role in the regulation of all intracellular physiological polyamines.

In summary, it was found that there is an Na^+/spermine antiporter in the rat renal brush-border membrane. This transporter recognizes straight-chain polyamine compounds that have more than 4 amino groups. This antiporter secretes intracellular spermine and regulates the intracellular spermine concentration. Furthermore, it plays an important role in the renal excretion of trientine.

Acknowledgement

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

References

Abdel-Monem, M. M., Merdink, J. L., Theologides, A. (1982) Urinary excretion of monoacetyl polyamines in patients with non-Hodgkin's lymphoma. Cancer Res. 42: 2097–2098

Biber, J., Stieger, B., Hasse, H., Murer, H. (1981) A high yield preparation for rat kidney brush–border membranes. Different behaviour of lysosomal markers. Biochim. Biophys. Acta 647: 169–176

Feuerstein, B. G., Pattabiraman, N., Marton, L. J. (1990) Molecular mechanics of the interactions of spermine with DNA: DNA bending as a result of ligand binding. Nucleic Acids Res. 18: 1271–1282

Fukumoto, G. H., Byus, C. V. (1996) A kinetic characterization of putrescine and spermidine uptake and export in human erythrocytes. Biochim. Biophys. Acta 1282: 48–56

Fukumoto, G. H., Byus, C. V. (1997) Putrescine export in Xenopus laevis oocytes occurs against a concentration gradient: evidence for a non-diffusional export process. Biochim. Biophys. Acta 1324: 215–222

Gawel-Thompson, K., Greene, R. M. (1988) Characterization of a polyamine transport system in murine embryonic palate mesenchymal cells. J. Cell. Physiol. 136: 237–246

Iseki, K., Kobayashi, M., Miyazaki, K. (1991) Spermine uptake by rat intestinal brush-border membrane vesicles. Biochim. Biophys. Acta 1068: 105–110

Kameji, R., Rannels, S. R., Pegg, A. E., Rannels, D. E. (1989) Spermidine uptake by type II pulmonary epithelial cells in primary culture. Am. J. Physiol. 256: C160–C167

Kashiwagi, K., Shibuya, S., Tomitori, H., Kuraishi, A., Igarashi, K. (1997) Excretion and uptake of putrescine by the PotE protein in *Escherichia coli*. J. Biol. Chem. 272: 6318–6323

Khan, N. A., Quemener, V., Moulinoux, J.-Ph. (1991) Polyamine membrane transport regulation. Cell Biol. Int. 15: 9–24

Kobayashi, M., Iseki, K., Saitoh, H., Miyazaki, K. (1992) Uptake characteristics of polyamines into rat intestinal brush-border membrane. Biochim. Biophys. Acta 1105: 177–183

Kobayashi, M., Tanabe, R., Sugawara, M., Iseki, K., Miyazaki, K. (1995) Sodium-dependent putrescine transport in rat intestinal basolateral membrane. Pharm. Sci. 1: 337–339

Kobayashi, M., Tanabe, R., Sugawara, M., Iseki, K., Miyazaki, K. (1997) The mechanism of excretion of trientine from rat kidney: trientine is not recognized by the H^+/organic cation transporter. J. Pharm. Pharmacol. 49: 426–429

Kurihara, H., Matsuzaki, S., Yamazaki, H., Tsukahara, T., Tamura, M. (1993) Relationship between tissue polyamine levels and malignancy in primary brain tumors. Neurosurgery 32: 372–375

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Murer, H., Hopfer, U., Kinne, R. (1976) Sodium/proton antiport in brush-border-membrane vesicles isolated from rat small intestine and kidney. Biochem. J. 154: 597–604

Pegg, A. E. (1986) Recent advances in the biochemistry of polyamines in eukaryotes. Biochem. J. 234: 249–262

Pegg, A. E., McCann, P. P. (1982) Polyamine metabolism and function. Am. J. Physiol. 243: C212–C221

Rowe, W. A., Tomicic, T. K., Hajjar, J. J. (1991) Enhancement of rat intestinal calcium absorption by vanadete. Proc. Soc. Exp. Biol. Med. 198: 754–759

Seiler, N. (1987) Functions of polyamine acetylation. Can. J. Physiol. Pharmacol. 65: 2024–2035

Seiler, N. (1990) Polyamine metabolism. Digestion 46 (Suppl. 2): 319–330

Seiler, N., Dezeure, F. (1990) Polyamine transport in mammalian cells. Int. J. Biochem. 22: 211–218

Seiler, N., Delcros, J. G., Moulinoux., J.-Ph. (1996) Polyamine transport in mammalian cells. Int. J. Biochem. Cell Biol. 28: 843–861

Sha, Q., Romano, C., Lopatin, N., Nichols, C. G. (1996) Spermidine release from Xenopus oocytes. Electrodiffusion through a membrane channel. J. Biol. Chem. 271: 3392–3397

Tabor, C. W., Tabor, H. (1976) 1,4-Diaminobutane (putrescine), spermidine, and spermine. Annu. Rev. Biochem. 45: 285–306

Takano, M., Inui, K., Okano, T., Saito, H., Hori, R. (1984) Carrier-mediated transport systems of tetraethylammonium in rat renal brush-border and basolateral membrane vesicles. Biochim. Biophys. Acta 773: 113–124

Takano, M., Inui, K., Okano, T., Hori, R. (1985) Cimetidine transport in rat renal brush–border and basolateral membrane vesicles. Life Sci. 37: 1579–1585

Takenoshita, S., Matsuzaki, S., Nakano, G., Kimura, H., Hoshi, H., Shoda, H., Nakamura, T. (1984) Selective elevation of the N^1-acetylspermidine level in human colorectal adenocarcinomas. Cancer Res. 44: 845–847

Van den Bosch, L., De Smedt, H., Missiaen, L., Parys, J. B., Borghgraef, R. (1990) Transport systems for polyamines in the established renal cell line LLC-PK: polarized expression of an Na(+)-dependent transporter. Biochem. J. 265: 609–612

Yung, M. W., Green, C. (1986) The binding of polyamines to phospholipid bilayers. Biochem. Pharmacol. 35: 4037–4041