Biochimica et Biophysica Acta, 727 (1983) 135–143
Elsevier Biomedical Press

BBA 71495

POLYCATIONS

A NEW CLASS OF INHIBITORS FOR IN VITRO SMALL INTESTINAL TRANSPORT OF SUGARS AND AMINO ACIDS IN THE RAT

BERND ELSENHANS [a,*], ROLAND BLUME [a], BERNHARD LEMBCKE [a], and WOLFGANG F. CASPARY [b]

[a] *Abteilung für Gastroenterologie und Stoffwechsel, Medizinische Klinik und Poliklinik, Universität Göttingen, Humboldtallee 1, 3400 Göttingen and* [b] *II. Medizinische Klinik, Stadtkrankenhaus Hanau, 6450 Hanau (F.R.G.)*

(Received March 26th, 1982)
(Revised manuscript received September 23rd, 1982)

Key words: Polycation; Amino acid transport; Sugar transport; Transport inhibitor; (Rat intestine)

Polycationic compounds like polylysine, protamine or polyethylenimine may interfere with a cation-related membrane transport system depending on superficially accessible binding sites for particular cations. In vitro experiments were performed using either everted segments of rat small intestine to measure tissue accumulation or everted sacs to determine mucosal-to-serosal transport. The effect of polycations was also tested using brush-border membrane vesicles of rat jejunum. Polycations inhibited the tissue accumulation of methyl α-D-glucoside as well as binding of phlorizin. Inhibition of accumulation was increased by raising the polycation concentration and by preincubation of the tissue with the polycations. Kinetic experiments revealed a competitive type of inhibition for the uptake of neutral amino acids and actively transported sugars. Using everted sacs to compare the monomeric cations with their corresponding polymeric forms for their inhibitory effect, it was found that only polymers applied to the mucosal compartment impaired active transport. The passive diffusion of solutes, e.g. 2-deoxy-D-glucose or mannitol, was slightly increased by polycations. With some intermediate oligomers of lysine it could be shown that more than 20 cationic groups are required for approximate complete inhibition. That membrane-related events are responsible for the observed inhibition is suggested by the reduced uptake of D-glucose by brush-border membrane vesicles in the presence of polycations. Therefore an interaction with transport-related cation binding sites, i.e. anionic residues, at the mucosal surface may be assumed.

Introduction

A variety of classes of compounds inhibit resorptive functions of the mucosa of the small intestine [1]. Commonly, inhibitory properties have been observed during research with analogs or as side-effects of drugs administered orally for therapeutic purposes, leaving the impairment of small intestinal transport for theoretical interests. The small intestinal effects of only a few classes of compounds have been found to be of therapeutic relevance, particularly biguanides [2] and sulfonylurea derivatives [3,4] which have been used for the oral treatment of diabetes mellitus. Recently, a physiological rather than molecular approach for

* To whom correspondence should be addressed.

Abbreviations: ACPC, 1-aminocyclopentane-1-carboxylic acid (cycloleucine); PEI, polyethylenimine; PDDP, poly[(*N*,*N*-dimethyl)-3,5-dimethylene-piperidinium chloride]; other abbreviations were used according to previously published recommendations, Eur. J. Biochem. 74, 1–6 (1977).

the inhibition of solute absorption from the small intestine has been proposed by investigators using carbohydrate gelling agents [5,6]. These viscosity-enhancing substances, i.e. neutral or anionic polysaccharides, did not directly interact with the membrane transport systems but increased the resistance of the unstirred diffusion barrier overlaying the absorptive surface of the small intestine.

In contrast to neutral or anionic polysaccharides, polycations adhere to the mucosal surface of the small intestine as it is clear from the structure and the composition of the brush-border membrane of the epithelial cells [7,8]. A binding to anionic membrane-bound residues appears very likely. If such negatively charged groups are important or even essential for Na^+-dependent transport systems, polycations should interfere with the active transport of sugars and amino acids. The aim of the present study is to characterize and to elucidate the effect of various polycations on the transport of these solutes by means of in vitro preparations of rat small intestine.

Methods and Materials

All studies were carried out using in vitro preparations of the jejunum from Wistar rats (Zentralinstitut für Versuchstiere, Hannover, F.R.G.) weighing 150–200 g. Non-fasted animals were sacrificed by stunning and decapitation and the mid jejunum was identified 10 cm beyond the duodenojejunal flexure. A 30–40 cm segment of jejunum was quickly excised, everted over a plastic rod and rinsed in chilled, oxygenated Krebs-Henseleit phosphate buffer.

Experiments with everted jejunal rings

These experiments were performed by incubations of rings of everted rat jejunum in vitro according to previously described and well-established methods [9–11]. Rings 0.5–1.0 cm in length were cut from the everted segment. Usually rings from two to four animals were pooled and randomized to minimize variability between animals and up to 10 rings were incubated in 25-ml Erlenmeyer flasks containing 5 ml of oxygenated (100% O_2) Krebs-Henseleit phosphate buffer at pH 7.2–7.4 and 37°C. Experiments were made in a shaking incubator (Model 3047, Köttermann, Hänigsen, F.R.G.) having a total horizontal excursion of about 1 cm at a rate of 120 cycles/min. Incubations were for 3 or 5 min since within this period tissue uptake was linear both in the controls and polycation-containing solutions. The short time period also minimized the influence of intracellular accumulation, reflecting mainly initial uptake rates required for the kinetic experiments.

The substrates (sugars and amino acids) were added to the incubation media as a mixture of radioactive tracer and unlabeled substance. In general, the media contained 0.01–0.02 μCi/ml for ^{14}C and 0.05–0.1 μCi/ml for ^{3}H. When the substrate concentration exceeded 5 mM, substrates were added at the expense of NaCl. When the polycations were added as free bases, the pH of the buffer had to be readjusted slightly by the addition of 0.1 mM HCl. However, the hydrochloride, hydrobromide or sulfate salts of the polycations were used where possible and caused negligible pH changes.

Binding of generally labeled [^{3}H]phlorizin (NEN Chemicals GmbH, Dreieich, F.R.G.) was also studied with the preparation described above. Using short-term incubations the effect of hydrolysis on the results by phlorizin hydrolase was minimized [12]. A kinetic study (unpublished results) without polycations revealed a binding constant of 0.6 μM, a value that agrees to previously published values of about 10^{-6} M [13]. Any error created by unspecific binding or uptake of radioactivity which would have shifted the binding constant to higher values, appears to be negligible.

Experiments with everted jejunal sacs

Everted sacs were prepared by a previously published method [14]. In general, four sacs, 6–7 cm in length were prepared from each animal and placed in chilled and oxygenated buffer. For a single absorption measurement 6 or 8 sacs were used, each taken from different rats and random distances from the duodenojejunal flexure. Sacs were filled with the appropriate medium (Krebs-Henseleit phosphate buffer when not otherwise stated), tied at both ends, and incubated for 35 min at 37°C in 25-ml Erlenmeyer flasks containing 10 ml medium. The media were composed as described for the ring experiments.

Membrane transport studies

Brush-border membranes of rat jejunum were prepared by a modified procedure [15] of a previously published method [16]. Mucosal scrapings were used instead of suspended epithelial cells. The uptake of D-[^{3}H]glucose by isolated brush-border membrane vesicles was measured by a rapid filtration technique [17].

Analytical methods and calculation of results

Processing of the incubated rings was accomplished by methods previously described [9]. After incubation, the everted sacs were removed, blotted, opened, and the sac fluid collected for analysis. When the tissue content of sugar or amino acids was desired the empty sac was handled by the same procedure as the rings. Either D-[^{14}C]- or D-[^{3}H]mannitol was used to correct for the extracellular space or to estimate passive diffusion. Radioactivity was assayed with a Searle liquid scintillation system (mark III) by using an automatic quench correction and a counting program for double-labeled samples.

Tissue accumulation or uptake of solute was expressed as a distribution ratio:

$$\text{Tissue uptake} = \frac{S_T}{S_M}$$

$$= \frac{\text{Solute concentration in the intracellular fluid volume}}{\text{Solute concentration in the incubation medium}}$$

assuming an intracellular fluid volume of 80% of the tissue net weight [9,11]. Tissue uptake rates for the evaluation of transport kinetics are expressed as the change in substrate concentration per minute in the intracellular fluid. To characterize the mucosal-to-serosal transport in sacs, the final substrate concentration is given. Membrane vesicular uptake was expressed as nanomoles (calculated from the specific activity of glucose in the medium) per mg of vesicular membrane protein. These values were corrected for nonspecific binding of radioactivity [16]. The unpaired Student's *t*-test was used to determine whether significant differences occurred in the indices measured.

Materials

1-Aminocyclopentane-1-[^{14}C]carboxylic acid (ACPC, cycloleucine), 2-deoxy-D-[^{3}H]glucose and D-[^{14}C]fructose were obtained from Amersham Buchler GmbH (Braunschweig, F.R.G.); all other radiochemicals were purchased from NEN Chemicals GmbH (Dreieich, F.R.G.). Protamine sulfate and pentalysine (Lys_5) were obtained from Serva (Heidelberg, F.R.G.), poly[(*N*,*N*-dimethyl)-3,5-dimethylenepiperidinium chloride] (PDDP) from EGA-Chemie (Steinheim, F.R.G.), polylysine (Lys_{8-12}) from Miles GmbH (Frankfurt, F.R.G.), and tetraethylenepentamine from Merck (Darmstadt, F.R.G.). From Sigma Chemie GmbH (München, F.R.G.) the following polycations were purchased: polyethylenimine (PEI), DEAE-dextran, Polybrene, and various poly(L-lysine)s (Lys_{20}, Lys_{60}, and Lys_{400}). Other materials were obtained from standard commercial sources.

Results

A variety of polycationic compounds tested in this study inhibited tissue accumulation of the actively transported sugar derivative methyl α-D-glucoside in vitro (Table IA). At a concentration of 2 mg/ml most of the polycations yielded a significant inhibition of the uptake of the sugar into everted jejunal rings. Only DEAE-dextran

TABLE I

IN VITRO SMALL INTESTINAL UPTAKE OF METHYL α-D-GLUCOSIDE AND BINDING OF PHLORIZIN; INHIBITION BY VARIOUS POLYCATIONS

Tissue uptake was determined in 5-min incubations which were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min using everted rings of rat jejunum. Polycation concentration was 2 mg/ml. Mean ± S.D. ($n = 8$), * $P < 0.001$. For further details see Methods.

	Tissue uptake (% of control)
A. Methyl α-D-glucoside (3 mM)	
Polylysine	47.9 ± 12.8 *
Polyarginine	49.7 ± 8.7 *
Polyornithine	44.9 ± 15.3 *
Protamine	76.7 ± 10.7 *
PDDP	38.2 ± 10.3 *
Polyethylenimine	37.2 ± 4.5 *
DEAE-dextran	90.8 ± 7.8
Polybrene	94.2 ± 11.6
B. Phlorizin (0.2 μM)	
Protamine	59,1 ± 8.5 *

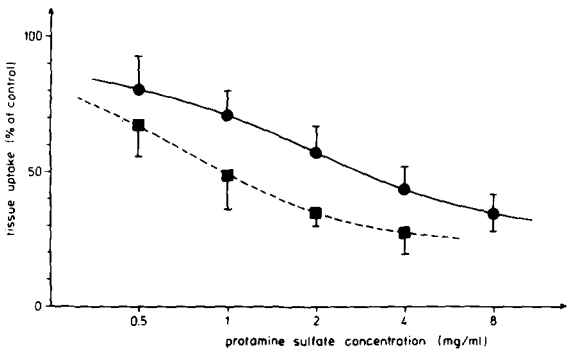

Fig. 1. Influence of protamine concentration and incubation conditions on the tissue uptake of methyl α-D-glucoside in rat small intestine in vitro. Incubations were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min using everted rings of rat jejunum, α-Me-Glc 3 mM. Uptake of methyl α-D-glucoside was determined after a 5-min incubation with (■- - - - -■) or without (●———●) a preceding 15-min preincubation without the solute added. Mean ± S.D. ($n = 6$).

and Polybrene caused a significantly lower but reproducible inhibition. Protamine not only inhibited the uptake of the sugar but also the binding of phlorizin to the carrier (Table IB).

As Fig. 1 shows, the effect of protamine on the tissue uptake of methyl α-D-glucoside was strongly dose dependent. With a 5-min incubation 50% inhibition was achieved with a protamine concentration of about 3 mg/ml. A 15-min pre-incubation period employing only the polycation followed by a short-term incubation (5 min) with sugar and polycation, again gave a clear dose dependency. Under these conditions a 50% inhibition was reached with a protamine sulfate concentration of about 1 mg/ml. Similar results were obtained for L-leucine uptake in the presence of protamine, polylysine, or polyethylenimine.

These three polycations were also compared for their effect on the development of the mucosal-to-serosal concentration gradient of actively and passively transported solutes (Table II). In everted sacs solutes and polycations were added only to the mucosal compartment since preliminary experiments (unpublished results) had revealed that polycations are only able to exhibit their inhibitory effect on the transport when they were applied to the mucosal side of this in vitro preparation. For the actively transported solutes (methyl α-D-glucoside, leucine, and cycloleucine) inhibition was very similar and amounted to about 50 to 70% with polyethylenimine as the slightly more effective inhibitor. In contrast to the Na^+-dependent active transport, the passive diffusion, exemplified by the transport of 2-deoxy-D-glucose, was significantly increased by all polycations tested. The transport of D-fructose, a compound transported by facilitated diffusion, was only slightly reduced by these polycations, significantly only in the case

TABLE II

INHIBITION OF MUCOSAL-TO-SEROSAL TRANSPORT OF DIFFERENT SOLUTES BY VARIOUS POLYCATIONS IN EVERTED SACS OF RAT JEJUNUM

Incubations were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min. Solutes and polycations (1 mg/ml) were added only to the mucosal compartment; solute concentration is given in parentheses. Mean ± S.D. ($n = 8$, except for fructose and 2-deoxy-D-glucose were sacs from six animals were used); * $P < 0.05$; ** $P < 0.005$; n.s. not significant. α-Me-Glc, methyl α-D-glucoside; Fru, D-fructose; dGlc, 2-deoxy-D-glucose; Leu, leucine; ACPC, cycloleucine.

Solute (mM)		Solute concentration in the serosal compartment (mM)					
		Polylysine		Protamine		Polyethylenimine	
		Control	+ polymer	Control	+ polymer	Control	+ polymer
α-Me-Glc	(3)	2.43 ± 0.87	1.17 ± 0.41 **	2.74 ± 0.46	1.58 ± 0.51 **	3.31 ± 0.73	1.14 ± 0.19 **
Fru	(3)	0.61 ± 0.14	0.49 ± 0.15 n.s.	0.61 ± 0.14	0.53 ± 0.05 n.s.	0.61 ± 0.14	0.46 ± 0.04 *
dGlc	(3)	0.25 ± 0.02	0.32 ± 0.04 **	0.25 ± 0.02	0.35 ± 0.04 **	0.25 ± 0.02	0.32 ± 0.05 *
Leu	(1)	0.96 ± 0.26	0.48 ± 0.13 **	1.22 ± 0.43	0.58 ± 0.27 **	1.06 ± 0.40	0.35 ± 0.04 **
ACPC	(1)	1.38 ± 0.32	0.55 ± 0.09 **	0.93 ± 0.23	0.34 ± 0.13 **	1.04 ± 0.24	0.24 ± 0.04 **

TABLE III

REQUIREMENT OF POLYMERIC STRUCTURE FOR IN VITRO INHIBITION OF SMALL INTESTINAL TRANSPORT

Incubations were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min for 35 min using everted sacs of rat jejunum. Mean ± S.D. ($n = 8$), * $P < 0.001$.

Mucosal solute (mM)	Solute concentration in the serosal compartment (mM)			
A	Cation derivative added (1 mg/ml)			
	Control	Ethylene-diamine	Tetraethylene-pentamine	Poly-ethylenimine
α-Me-Glc (3)	3.78 ± 0.66	3.53 ± 0.72	3.67 ± 1.04	1.21 ± 0.37 *
ACPC (1)	0.98 ± 0.21	1.07 ± 0.27	1.15 ± 0.20	0.27 ± 0.05 *
B	Cation derivative added (1 mg/ml)			
	Control	Lysine	Pentalysine	Polylysine
ACPC (1)	0.90 ± 0.28	0.80 ± 0.22	0.80 ± 0.20	0.38 ± 0.13 *

of the more potent polyethylenimine.

Inhibition by polycations could have been due, at least in part, to the action of intermediates or degradation products, e.g. monomers. Therefore, everted sacs were used to compare the polycations polylysine and polyethylenimine with corresponding short-chain derivatives and monomers for their effect on the development of the mucosal-to-serosal concentration gradient of methyl α-D-glucoside and cycloleucine. As Table III clearly shows, the only significant effect was observed when the polycations were applied in their polymeric form in the mucosal compartment of the preparation. Other conditions, e.g. application of the compounds at the serosal side, did not reveal any effect on the transmural solute movements. The same observations were made comparing polyornithine and polyarginine with their monomers.

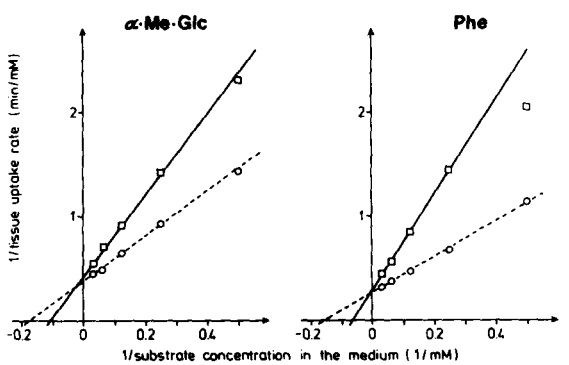

Fig. 2. Effect of PDDP on the in vitro transport kinetics of methyl α-D-glucoside (α-Me-Glc) and phenylalanine (Phe) in rats jejunum. Tissue uptake rates were determined in the presence (□———□) or absence (○- - - - -○) of PDDP (2 mg/ml) in the medium. The 3-min incubations were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min using everted jejunal rings. Results are means of four separate experiments and are plotted according to Lineweaver-Burk.

Kinetic experiments were performed using everted rings. Various amino acids (leucine, phenylalanine) and sugars (glucose, methyl α-D-glucoside, galactose) were employed together with different polycations (protamine, polyethylenimine, PDDP) in short-term incubations. The results obtained with PDDP (Fig. 2) are representative for all the other combinations of solute and polycation. All these experiments equally showed an increase in the apparent transport K_m as the inhibitory effect of the polycations (plots according to Lineweaver-Burk or Eadie-Hofstee gave the same results). It is noteworthy that the K_m increment was essentially independent of the agitation of the tissue although it tended to increase slightly with higher shaking rates (> 250 cycles/min).

Additionally, kinetic experiments were carried

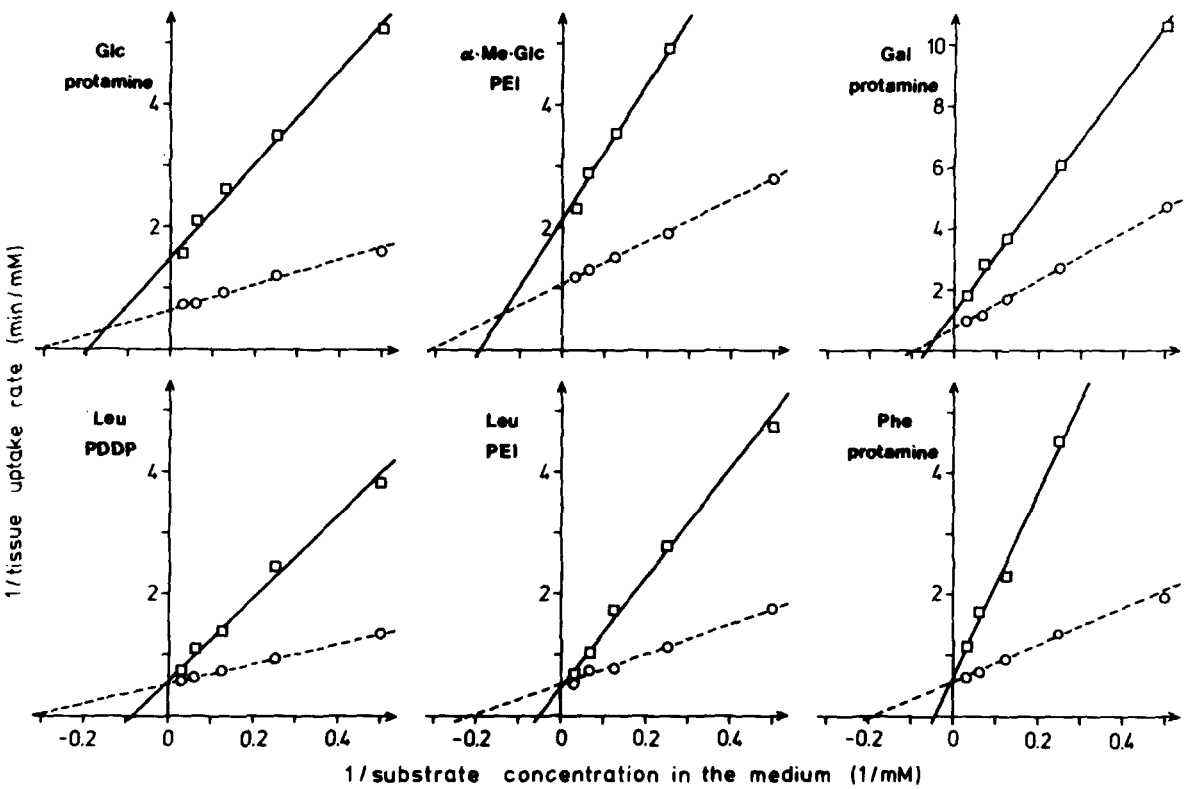

Fig. 3.Effect of a preincubation period with various polycations on the in vitro transport kinetics of different sugars and amino acids. Tissues were incubated for 23 min (with (□———□) or without (O- - - - -O) polycation, 1 mg/ml). During the last 3 min substrates were added to both control and test tissue incubations.

out after having preincubated the tissue in media containing polycations. Most strikingly, polycations differentially inhibited actively transported sugars and amino acids (Fig. 3). Whereas the amino acids again were influenced by a competitive type of inhibition, polycations exerted a mixed type of inhibition in the case of the monosaccharides. This observation was very consistent and, as above in the experiments without a preincubation step, not essentially altered by variations in the shaking rate.

As shown in Table III, the polycation-mediated inhibition depended on the intact polymeric form. Therefore, experiments were performed with commercially available products to study the effect of polylysines of different chain length. As depicted in Table IV, the inhibition became significant with lysine derivatives greater than 20 residues in length. The passive diffusion, exemplified by the transport of mannitol, was affected in a quite opposite manner. Assuming the same diffusion properties for both solutes under these conditions, the inhibition of the active transport component can be estimated to 85% for Lys_{60}.

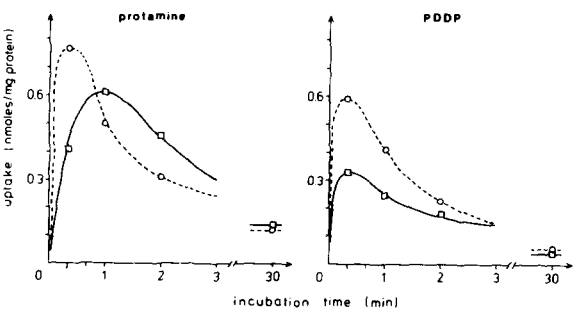

Fig. 4. Inhibition of the uptake of D-glucose into jejunal brush-border membrane vesicles by polycations. O- - - - -O, control incubation; ■———■, test incubation with a polycation concentration of 2 mg/ml; D-glucose concentration was 50 μM. Results are means of two separate experiments in duplicate.

TABLE IV

INFLUENCE OF INCREASING CHAIN LENGTH OF POLYLYSINES ON THEIR INHIBITORY EFFECT ON THE MUCOSAL-TO-SEROSAL TRANSPORT OF METHYL α-D-GLUCOSIDE IN EVERTED SACS OF RAT JEJUNUM

Incubations were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and 120 cycles/min for 35 min. Solutes and lysines were added only to the mucosal compartment. Initial concentrations of methyl α-D-glucoside and mannitol were 3 and 1 mM, respectively; concentration of lysines was 1 mg/ml. Mean ± S.D. ($n = 6$), ** $P < 0.005$, significances are related to the control incubation where no lysine was added.

Lysine derivative	Solute concentration in the serosal compartment (mM)	
	α-Me-Glc	Mannitol
–	3.66 ± 1.13	0.06 ± 0.01
Lysine	3.73 ± 1.64 n.s.	0.08 ± 0.02 n.s.
Lys_5	3.38 ± 1.07 n.s.	0.08 ± 0.05 n.s.
Lys_{8-12}	2.78 ± 1.04 n.s	0.13 ± 0.02 **
Lys_{20}	1.09 ± 0.36 **	0.15 ± 0.04 **
Lys_{60}	0.96 ± 0.38 **	0.15 ± 0.02 **

Experiments were also carried out using brush-border membrane vesicles of rat jejunum. The results shown in Fig. 4 clearly demonstrate that polycations are able to impair the overshoot phenomenon characteristic for the uptake of glucose into these vesicles in the presence of an Na^+ gradient. The differences between protamine and PDDP concerning their effect on the transport across the membrane were not further investigated but may be due to different reactivities of the two polycations towards the vesicle surface.

Discussion

The present investigation clearly demonstrates that polycations are capable of inhibiting Na^+-dependent solute uptake in the small intestine of rats. A variety of polycations were tested showing that not all were equally active. Fig. 5 lists the chemical structures of the polycations used in the present study. From the structure of these polycations and their corresponding interactions it is tentatively concluded that polycations with sterically hindered and hence less accessible amino groups, e.g. Polybrene and DEAE-dextran, are less effective in the inhibition of Na^+-dependent solute uptake.

That the observed inhibition was not primarily due to intracellular events but could be due to polycation-interactions with the luminal membrane surface of the small intestine was suggested in the present study by the effect of protamine on the binding of phlorizin. This view was strengthened by the present findings with brush-border membrane vesicles where the overshoot phenomenon was clearly reduced. Further support may be obtained from investigations in which polycations were used to inhibit membrane-related functions in various preparations, e.g. the ion transport across mitochondrial membranes [18,19], or the photosystem activities of isolated chloroplasts [20] which suggested electrostatic interactions between the polycations and the membranes. It is also well known that alterations in the surface charge of membranes affect their passive and energy-linked permeability to ions [21,22] and metabolites [23, 24].

Reports of destabilization by polycations of the microvillous membrane in rabbit gallbladder [25] or the disruption of the luminal cell membranes in the kidney proximal tubule [26] might be organ or species specific. Recent morphological studies [8] about anionic sites on the brush border of rat small intestine, using polylysine and incubation conditions similar to those described in this study, did not reveal any alteration in the morphology of the microvillous membrane.

From the present results, freely accessible, fixed negative charges on the brush border of the small intestinal absorptive cells appear to be essential for optimal functioning of the active and Na^+-dependent transport processes. External Na^+ is one of the main requirements of Na^+-dependent active influx of nonelectrolytes [27]. This is supported by the fact that Mg^{2+} was more effective than mannitol in reducing sugar uptake during small intestinal perfusions of Na^+-free media [28]. In these experiments Mg^{2+} was probably able to displace residual, secreted Na^+ from those fixed negative charges.

No matter whether an ion-exchange region, specific for Na^+, is operative [29] or whether the brush border is merely a trap for all kinds of cations, it should be possible to alter the cationic

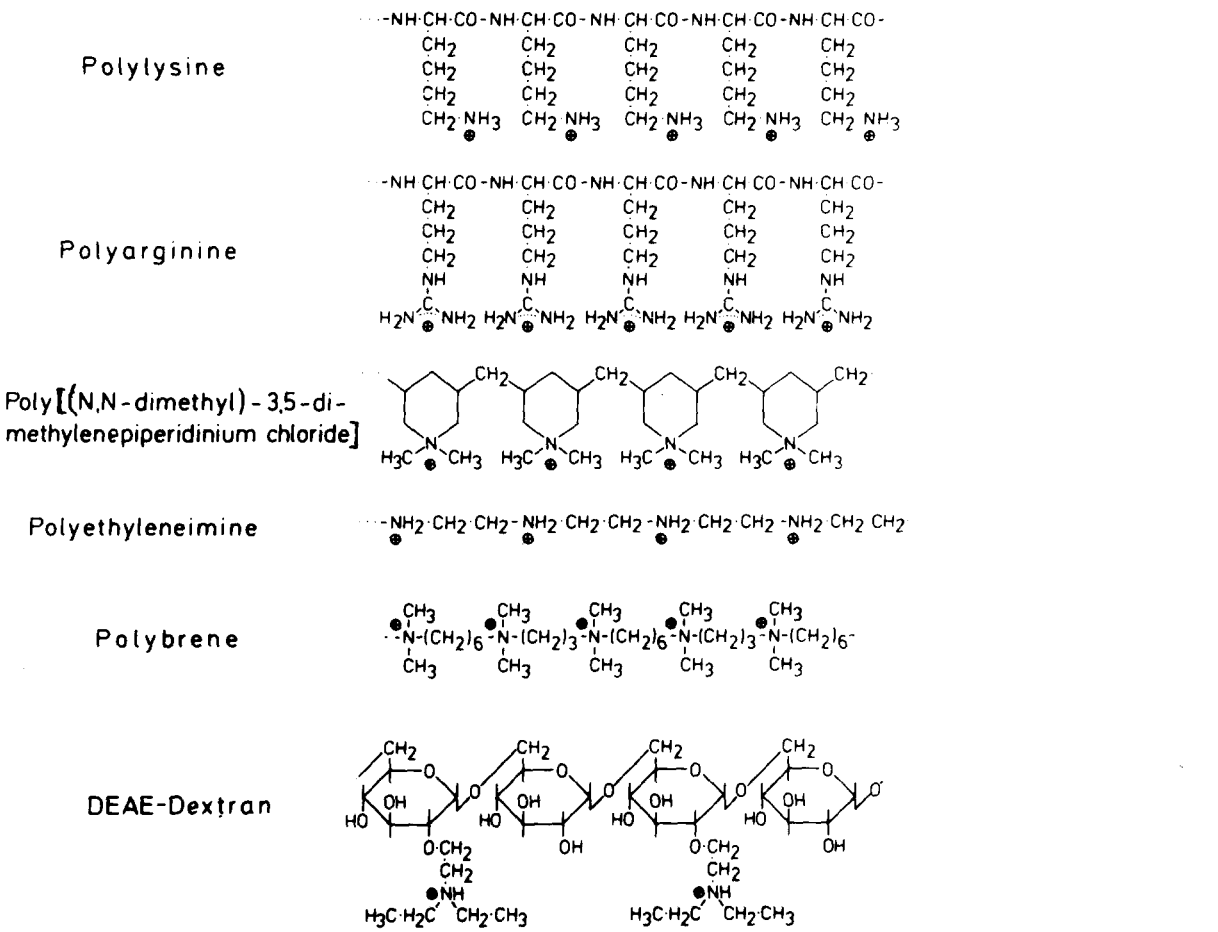

Fig. 5. Structure of polycations.

microenvironment at the luminal cell surface with polyvalent cations [30]. The present investigation employing different polylysines show that a certain number of cationic residues are required to block efficiently the Na^+-dependent solute uptake. This probably depends on the affinity of the polycation for the polyanionic matrix of the brush-border membrane. Therefore, the chain length for equal inhibition of the active transport may differ for the various polycations. From the nature of the reaction between positively and negatively charged polyelectrolytes [31] it was expected that under physiological electrolyte concentrations a reversibility could not be achieved. This was confirmed in an experiment which showed treatment of the tissue with a negatively charged polymer, poly(galacturonic acid), did not significantly restore the uptake of methyl α-D-glucoside after the inhibition by polylysine (unpublished results).

From the present results it is not yet clear whether the polycations block Na^+-binding sites of the transport carriers, or cation-binding sites of more distally located groups of the glycocalyx, or perhaps both. The results obtained from the present kinetic experiments (Na^+-rich media + polycations) resemble those studies in which Na^+-free media were employed [11,32,33]. Definitely, this analogy does not provide very strong evidence for the conclusion that the presence of polycations may displace Na^+, necessary for active transport, but may support this idea.

The differential inhibition of the uptake of amino acids and monosaccharides after a preincubation period with the polycations was not further investigated. Assuming a similar Na^+-

dependency for both transport systems, one could state that the prolonged effect of the polycations preferentially alters the number of the carriers available for sugar transport. Since the epithelial tissue swells when treated with transport inhibitors in the presence of Na^+ [34], this may also contribute to the present observations, particularly if sugar and amino acid carriers do not share the same distribution pattern along the villous. The enhancement of the passive diffusion is probably due to an increase in the small intestinal permeability, in analogy to observations of increased serosal-to-mucosal fluxes of alanine in an Na^+-free medium [35].

In conclusion, certain polycations inhibit Na^+-dependent uptake of organic solutes in the small intestine of rats. Definitely, this inhibition depends on the intact polymeric form. The present findings suggest an interaction between the polycations and transport-related cation binding sites at the membrane surface resulting in a displacement of Na^+ from the transport carrier or its vicinity.

Acknowledgements

This work was supported by the grants Ca 71/11 and Le 468/1 from the Deutsche Forschungsgemeinschaft.

References

1 Caspary, W.F. (1977) Dtsch. Med. Wochenschr. 102, 167–173
2 Caspary, W.F. (1977) Acta Hepato-Gastroenterol. 24, 473–480
3 Teale, J.D. and Love, A.H.G. (1972) Biochem. Pharmacol. 21, 1839–1848
4 Teale, J.D. and Love, A.H.G. (1973) Biochem. Pharmacol. 22, 997–1004
5 Elsenhans, B., Süfke, U., Blume, R. and Caspary, W.F. (1980) Clin. Sci. 59, 373–380
6 Johnson, I.T. and Gee, J.M. (1981) Gut 22, 398–403
7 Dobbins, W.O., III (1969) Am. J. Med. Sci. 258, 150–171
8 Jersild, R.A., Jr. and Crawford, R.W. (1978) Am. J. Anat. 152, 287–306
9 Crane, R.K. and Mandelstam, P. (1960) Biochim. Biophys. Acta 45, 460–476
10 Caspary, W.F., Stevenson, N.R. and Crane, R.K. (1969) Biochim. Biophys. Acta 193, 169–178
11 Alvarado, F. and Mahmood, A. (1974) Biochemistry 13, 2882–2890
12 Warden, D.A., Fannin, F.F., Evans, J.O., Hanke, D.W. and Diedrich, D.F. (1980) Biochim. Biophys. Acta 599, 664–672
13 Alvarado, F. (1967) Biochim. Biophys. Acta 135, 483–495
14 Wilson, T.H. and Wiseman, G. (1954) J. Physiol. Lond. 123, 116–125
15 Schmitz, J., Preiser, H., Maestracci, D., Gosh, B.K., Cerda, J.J. and Crane, R.K. (1973) Biochim. Biophys. Acta 323, 98–112
16 Lücke, H., Berner, W., Menge, H. and Murer, H. (1978) Pflügers Arc. 373, 243–248
17 Berner, W., Kinne, R. and Murer, H. (1976) Biochem. J. 160, 467–474
18 Schwartz, A. (1965) J. Biol. Chem. 240, 939–943; 944–948
19 Johnson, C.L., Mauretzen, C.M., Starbuck, W.C. and Schwartz, A. (1967) Biochemistry 6, 1121–1127
20 Brand, J., Baszynski, T., Crane, F.L. and Krogmann, D.W. (1972) J. Biol. Chem. 247, 2814–2819
21 Huunan-Seppälä, A. (1971) Bioenergetics 2, 197–207
22 Schäfer, G. (1976) Biochem. Pharmacol. 25, 2005–2014
23 Meisner, H., Palmieri, F. and Quagliariello, E. (1972) Biochemistry 11, 949–955
24 Kessler, M., Meier, W., Storelli, C. and Semenza, G. (1975) Biochim. Biophys. Acta 413, 444–452
25 Quinton, P.M. and Philpott, C.W. (1973) J. Cell Biol. 56, 787–796
26 Sato, K. and Ullrich, K.J. (1975) J. Membrane Biol. 21, 311–321
27 Curran, P.F. (1975) in Intestinal Absorption and Malabsorption (Csáky, T.Z., ed.), pp. 113–126, Raven Press, New York
28 Fordtran, J.S. (1975) in Inetstinal Absorption and Malabsorption (Csáky, T.Z., ed.), pp. 229–235, Raven Press, New York
29 Alvarado, F. (1976) in Intestinal Ion Transport (Robinson, J.W.L., ed.), pp. 117–152, MTP Press, Lancaster
30 Theuvenet, A.P.R. and Borst-Pauwels, G.W.F.H. (1976) Biochim. Biophys. Acta 426, 745–756
31 Scott, J.E. (1973) Biochem. Soc. Trans. 1, 787–806
32 Crane, R.K., Forstner, G. and Eichholz, A. (1965) Biochim. Biophys. Acta 109, 467–477
33 Curran, P.F., Schultz, S.G., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1261–1286
34 Schultz, S.G., Fuisz, R.E. and Curran, P.F. (1966) J. Gen. Physiol. 49, 849–866
35 Hajjar, J.J., Khuri, R.N. and Bikhazi, A.B. (1975) Am. J. Physiol. 229, 518–523