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In vitro inhibition of rat small intestinal absorption by lipophilic organic cations

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Cationic, lipid-soluble organic compounds may interfere with cation-mediated membrane transport processes. Thus, small intestinal absorption may be influenced by lipophilic organic cations. Therefore a series of arylalkylamines was studied in the concentration range from 0.5 to 20 mmol/l for their effect on the transport of various monosaccharides and leucine in the rat small intestine in vitro by means of the tissue accumulation technique. Whereas the monophenyl substituted monoamines (e.g. benzylamine, 2-phenylethylamine, 3-phenylpropylamine) did not show a significant effect on the active transport, the corresponding ω,ω-diphenyl derivatives exhibited a strong inhibition of the active transport of the sugars and the amino acid. These monoamines and drugs of similar structure (e.g. benzoctamine, diphenhydramine) exhibited a mixed or non-competitive type of inhibition which correlated quite well with their octanol-water partition coefficients. In contrast, di- or triamines (e.g. harmaline, imipramine, pyrilamine) revealed a rather pure competitive type of inhibition. These findings tentatively suggest a different mode of action on the active transport by lipid-soluble organic amines according to the molecular charge distribution. In addition, membrane vesicles were used to examine the effect of the different amines on the sucrase activity. Regarding the cation-dependent hydrolysis of sucrose, however, no distinct pattern developed.

Introduction

Studying the effects of agents interacting with membranes may contribute to the understanding of the mechanisms of solute translocation across biological membranes. Lipid-soluble, organic cations may be embedded into the lipid matrix of the membrane or bound to membrane phospholipids, thus affecting membrane fluidity and potentials [1]. They also may compete with other cations for the binding to transport-related cation-binding sites.

At the mucosal surface of the small intestine, cation-dependent mechanisms are important for the digestion and absorption of nutrients. Both, sucrose hydrolysis by the brush-border sucrase and active transport of sugars and amino acids by the brush-border carriers were found to be activated by sodium ions. Therefore it was conceivable that other cations may interfere with these processes. In fact, various cationic compounds impair sucrase activity and active transport in the small intestine. It was shown that sucrase is inhibited by Tris [2] and harmaline [3], and that active transport can be impaired by compounds such as biguanides [4], harmaline [5], prenylamine [6], and chlorpromazine [7]. Lipophilic interactions between membrane and cationic agent are thought

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to be at least partially responsible for reducing the Na^+-dependent transport. That cationic compounds may act directly with anionic residues at the luminal side of the mucosal surface and thus may impair active transport was suggested by a recent study employing polycations [8].

Due to their great structural variety, organic amines may provide a useful tool to investigate possible effects of different cations on the absorptive and digestive functions of the small intestinal mucosa. The present study is concerned with a characterization of the effect of some arylalkylamines on rat small intestinal absorption in vitro.

Methods and Materials

In vitro uptake of ^{14}C-labeled solute was measured by the tissue accumulation technique [9] using everted rings of the small intestine of rats (jejunum from non-fasted female Wistar rats, 150–200 g; Zentralinstitut für Versuchstiere, Hannover, F.R.G.) as recently described in detail [8]. The incubations were carried out in oxygenated (100% O_2) Krebs-Henseleit phosphate buffer (pH 6.5). D[^{3}H]Mannitol together with unlabeled mannitol (1 mmol/l) was used to correct for the extracellular space. Tissue accumulation or uptake of the 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 [10]. The tissue uptake rates for the evaluation of transport kinetics are expressed as the change in the intracellular solute concentration per minute.

For the kinetic uptake experiments the incubations were carried out for 5 min since within this period the tissue uptake was linear both in the controls and amine containing solutions. This short time period also minimizes the influence of intracellular accumulation on the results, thus reflecting mainly initial uptake rates which were required for the kinetic studies. When experiments were performed in buffer solutions of reduced Na^+ concentrations the corresponding amount of NaCl removed at the preparation of the buffer was substituted by the addition of mannitol.

Intracellular sodium and potassium concentrations were determined in everted rings after 5-min incubations during which the conditions were those of the kinetic uptake experiments. The measurements were carried out according to a previously published method [11] using an internal standard flame photometer (type FCM 6341, Eppendorf, Hamburg, F.R.G.).

Sucrose hydrolysis was carried out using brush-border membrane vesicles prepared from rat jejunum by established procedures [12]. Sucrase activities were determined by the Tris-glucose oxidase method [2]. Of the compounds used, only harmaline interfered with the determination of the glucose [3]. In this case, the glucose measurements were corrected using glucose standard curves at the various harmaline concentrations (1, 2, 3, and 5 mmol/l).

The octanol-water partition coefficients were measured by shaking the aqueous amine solutions (1 mmol/l in Krebs-Henseleit phosphate buffer, pH 6.5) with an equal volume of *n*-octanol at 20°C for 24 h to warrant an equilibrium distribution. In both phases the amine concentrations were measured spectrophotometrically. The concentration ratio octanol : buffer was considered as the octanol-water partition coefficient.

Except of imipramine and pyrilamine which were purchased from Sigma Chemie GmbH (München, F.R.G.), and of diphenhydramine (ICN-K & K Laboratories Inc., Plainview, NY, U.S.A.) and benzoctamine (a gift from Ciba Geigy GmbH, Wehr, F.R.G.), the amines tested in this study were obtained from EGA-Chemie (Steinheim, F.R.G.). Radiochemicals were purchased from NEN Chemicals GmbH (Dreieich, F.R.G.); other materials were obtained from standard commercial sources.

Results

Various arylalkylamines were found to inhibit the tissue accumulation of the actively transported sugar derivative methyl α-D-glucoside (Table I). Particularly the amines containing two aromatic rings (either single-bonded or condensed to tricyclic systems) caused a marked inhibition of the

TABLE I

INHIBITION OF SMALL INTESTINAL UPTAKE OF METHYL α-D-GLUCOSIDE BY VARIOUS ARYLALKYLAMINES

The sugar uptake was measured by 10-min incubations in oxygenated Krebs-Henseleit phosphate buffer at 37°C and a shaking rate of 120 cycles/min with everted jejunal rings. Amine concentration: 10 mmol/l, sugar concentration: 1 mmol/l; values are means of four separate determinations (mean ± S.D.); control value: 100 ± 15; * $P < 0.05$, ** $P < 0.001$ (related to the control).

Compound	Uptake (% of control)	Compound	Uptake (% of control)
Methylamine	103 ± 10	1,1-Diphenylmethylamine	46 ± 8 **
n-Pentylamine	98 ± 11	2,2-Diphenylethylamine	21 ± 2 **
Aniline	106 ± 9	3,3-Diphenylpropylamine	9 ± 1 **
Benzylamine	97 ± 12	2,2-Diphenylpropylamine	18 ± 3 **
2-Phenylethylamine	98 ± 7	1,3-Diphenyl-2-aminopropane	28 ± 3 **
3-Phenylpropylamine	94 ± 9	Diphenhydramine	25 ± 7 **
4-Phenylbutylamine	74 ± 8 *	Benzoctamine	8 ± 3 **
Diphenylamine	101 ± 6	Pyrilamine	30 ± 12 **
Dibenzylamine	36 ± 12 **	Imipramine	4 ± 1 **
9-Aminofluorene	24 ± 3 **	Harmaline	32 ± 5 **

sugar uptake into the tissue. Unsubstituted aliphatic amines, e.g. methylamine and pentylamine, had no effect and the longer chained monophenyl derivatives were only weak inhibitors. In addition, the aromatic amines, aniline and *N*,*N*-diphenylamine did not cause any significant inhibition under these experimental conditions.

Applying increasing inhibitor concentrations under the conditions described for the experiment depicted in Table I the inhibition of the uptake of methyl α-D-glucoside (1 mmol/l) was strongly dose-dependent (Fig. 1). The amine concentrations needed to achieve 50% inhibition of the control uptake ranged from 11 mmol/l for 1,1-diphenylmethylamine to 3.4 mmol/l for benzoctamine; the monophenyl derivatives were not included in these experiments due to the rather high concentrations (above 40 mmol/l) needed to produce a 50% inhibition.

For a further elucidation of the inhibition, kinetic experiments were performed (Fig. 2). The monoamines, the ω,ω-diphenylalkylamines, benzoctamine, diphenhydramine, and 9-aminofluorene, exhibited a noncompetitive or mixed type of inhibition. The decrease in the maximal transport capacity by this group of inhibitors appeared to be dependent on the length of the aliphatic chain. In contrast, a competitive type of inhibition was found for the di- or triamines (harmaline, pyrilamine and imipramine) employed in these experiments.

Only under certain conditions, inhibition was found to be reversible. At low inhibitor concentrations (< 2–3 mmol/l) and short incubation periods (< 2–5 min) washing of the tissue restored the uptake of methyl α-D-glucoside. This was exemplified by experiments with harmaline and 3,3-diphenylpropylamine (Table II). The higher the amine concentration and the longer the exposure of the tissue to the inhibitors the smaller was the portion of uptake which appeared to be reversible.

Several compounds were selected to study also the influence of the amines on the small intestinal

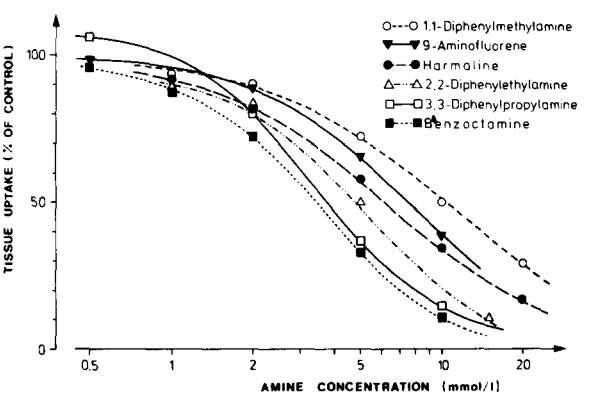

Fig. 1. Concentration-dependent inhibition of jejunal uptake of methyl α-D-glucoside (1 mmol/l) by various organic amines. Incubation conditions were those depicted in the legend for Table I.

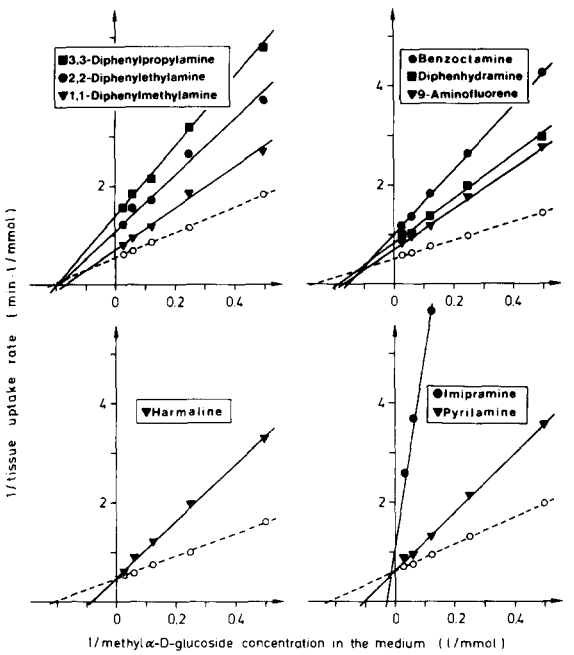

Fig. 2. Effect of various organic amines (8 mmol/l) on the in vitro transport kinetics of methyl α-D-glucoside in rat jejunum. Tissue uptake rates were determined in the presence (———) or absence (- - - -) of the inhibitors in the medium. The 5-min incubations were carried out in oxygenated Krebs-Henseleit 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.

uptake of differently transported solutes (Table III). A strong inhibition was observed for actively transported amino acids (leucine) and sugars (galactose). However, facilitated (fructose) and passive diffusion (2-deoxyglucose) was not affected. With benzoctamine as one of the strongest and with harmaline as a somewhat weaker inhibitor of the active transport of leucine and galactose, the relative ability of the amines to inhibit active transport was very similar to the pattern obtained for the inhibition of the uptake of methyl α-D-glucoside (see Table I).

In addition, kinetic experiments were performed to evaluate the effect of several amines on the active transport at two different Na^+ concentrations (60 and 120 mmol/l). With a constant concentration of methyl α-D-glucoside (1 mmol/l) increasing concentrations of the inhibitors were employed. The results obtained for diphenhydramine and pyrilamine (Fig. 3) are representative for the effect of the other amines tested (harmaline, 3,3-diphenylpropylamine, and benzoctamine). Using the Dixon plot, Na^+ can be regarded as the substrate for the process inhibited by the amines. Thus, the intercept between the two lines obtained for the two different Na^+ concentrations lying above the abscissa demonstrates a competitive inhibition of Na^+ by the amines. Therefore, the relative effectiveness of the amines increased at the lower Na concentration; e.g. at the high Na^+ concentration an addition of diphenhydramine (7.5 mmol/l) reduced the uptake of methyl α-D-glucoside to about 46% and at the lower Na^+ concentration further to about 36% that of the control.

Interestingly, the determination of the intracellular Na^+ and K^+ concentrations did not reveal significant changes after adding the amines to the incubation medium. The values obtained ranged between 80 and 100 mmol/l for potassium and between 50 and 70 mmol/l for sodium.

Regarding the inhibitory effect of the amines investigated in the present study, a tentative correlation between the octanol-water partition coefficients and the inhibition of methyl α-D-glucoside uptake may be postulated (Fig. 4).

In brush-border membrane vesicles the specific sucrase activity was enriched about 40-fold with

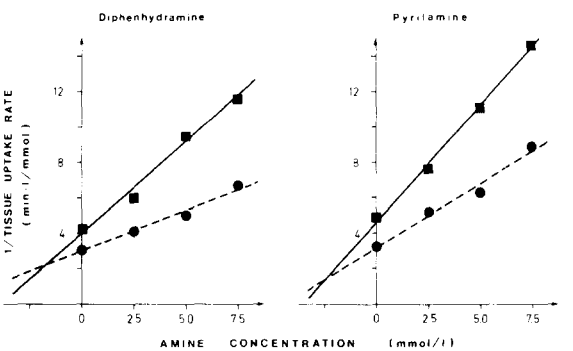

Fig. 3. Influence of two different Na^+ concentrations on the inhibitory effect of diphenhydramine and pyrilamine as measured by the jejunal tissue uptake of methyl α-D-glucoside (Dixon plots). The 5-min incubations were carried out at sodium concentrations of 120 mmol/l (●- - - -●) and 60 mmol/l (■———■) employing 37°C and a shaking rate of 120 cycles/min. Methyl α-D-glucoside concentration was 1 mmol/l and the results are means of four separate experiments.

TABLE II

REVERSIBILITY OF THE EFFECT OF HARMALINE AND 3,3-DIPHENYLPROPYLAMINE ON THE JEJUNAL UPTAKE OF METHYL α-D-GLUCOSIDE

After the various preincubation periods with (+inhibitor) or without (control) amine added, the tissue was washed with 20 ml of Krebs-Henseleit phosphate buffer at 37°C with (tissue treated) or without (control; tissue washed) inhibitor added. Subsequently tissue uptake of methyl α-D-glucoside (1 mmol/l) was determined (5-min incubation; everted jejunal rings; Krebs-Henseleit phosphate buffer) in the presence (tissue treated) and absence (control; tissue washed) of the amine. Mean ±S.D. ($n = 4$). * $P < 0.05$; ** $P < 0.005$ (related to the control, Student's t-test).

Inhibitor (mucosal concentration)	Preincubation period (min)	Tissue uptake (S_T/S_M)				
		Control	+Inhibitor			
			Tissue treated	% of control	Tissue washed	% of control
Harmaline:						
3 mmol/l	2	1.60±0.32	1.05±0.10 *	66	1.67±0.07	104
	5	2.69±0.31	1.55±0.25 **	58	2.26±0.25	84
	10	2.81±0.43	1.69±0.12 **	60	2.30±0.07	82
5 mmol/l	3	1.38±0.26	0.47±0.12 **	34	0.89±0.41	65
3,3-Diphenylpropylamine:						
3 mmol/l	2	1.52±0.13	0.65±0.21 **	43	1.26±0.19	83
	5	1.98±0.19	0.73±0.08 **	37	1.56±0.08 *	79
	10	2.83±0.40	0.65±0.24 **	23	1.40±0.17 **	50
5 mmol/l	3	2.17±0.35	0.57±0.10 **	26	1.59±0.20 *	74

respect to the mucosal homogenate. With this preparation the effect of 3,3-diphenylpropylamine and pyrilamine (two compounds thought to be representative for the amines tested in this study) on sucrase activity was compared with that of harmaline. As expected, harmaline inhibited sucrase competitively and showed an inhibitor constant ($K_i \sim 2$ mmol/l) almost identical to the one reported previously [3]. However, 3,3-diphenylpropylamine and pyrilamine exhibited a different pattern (Fig. 5). Whereas pyrilamine activated sucrose hydrolysis at concentrations up to 9 mmol/l, 3,3-diphenylpropylamine essentially caused inhibition except of a slight activation at low concentrations

TABLE III

INHIBITION OF SMALL INTESTINAL UPTAKE OF DIFFERENT SOLUTES BY VARIOUS AMINES

Tissue uptake was determined in 5-min incubations which were carried out in oxygenated Krebs-Henseleit phosphate buffer at 37°C and a shaking rate of 120 cycles/min using everted rings of rat jejunum. The values are means of three separate experiments in duplicate. Mean ±S.D. ($n = 3$) * $P < 0.05$, significance is related to the control incubation where no amine was added (Student's t-test).

Solute (0.1 mmol/l)	Tissue uptake (S_T/S_M)				
	Control	+Inhibitor (8 mmol/l)			
		3,3-Diphenylpropylamine	Harmaline	Benzoctamine	Pyrilamine
Leu	3.46±0.45	1.72±0.16 *	2.39±0.36 *	1.67±0.26 *	2.01±0.14 *
Gal	1.61±0.08	0.50±0.07 *	0.67±0.10 *	0.35±0.06 *	0.69±0.08 *
Fru	0.24±0.05	0.29±0.04	0.23±0.05	0.27±0.03	0.21±0.04
dGlc	0.24±0.03	0.25±0.06	0.24±0.05	0.24±0.03	0.24±0.04

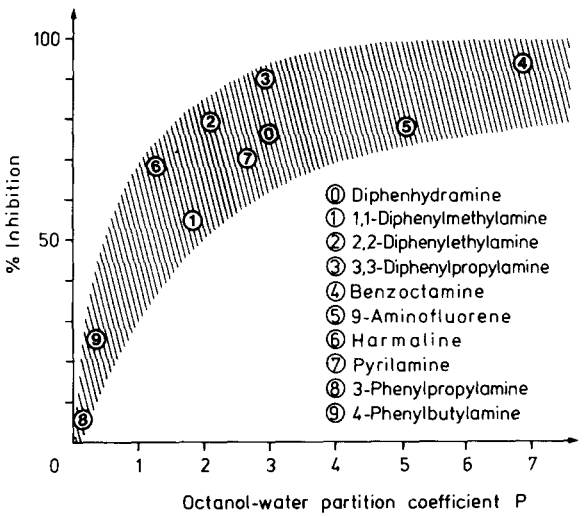

Fig. 4. Correlation between the inhibitory effect and the octanol-water partition coefficient of various organic amines. Inhibition of the uptake of methyl α-D-glucoside was measured under the conditions described in the legend for Table I.

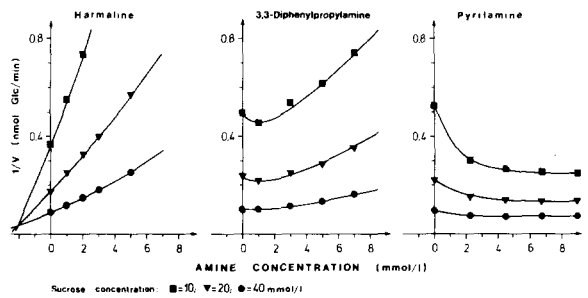

Fig. 5. Influence of some selected organic amines on the hydrolysis of sucrose by rat jejunum (Dixon plots). The hydrolysis was carried out using brush-border membrane vesicles of rat jejunum. 20-min incubations at 37°C and pH 6.8; buffer: isobutylammonium maleate (10 mmol/l) and mannitol (110 mmol/l). Results are means of two separate experiments in triplicate.

(≤ 1 mmol/l). Regarding only the effect at higher concentrations (3–7 mmol/l), a competitive type of inhibition may be assumed ($K_i \sim 6$ mmol/l).

Discussion

Organic amines are certainly able to interfere with Na^+-dependent functions of the small intestinal brush border. As indicated in this study, the effect on the active transport correlated to some extent with the lipophilic properties of the organic cations. As the main structural features of the inhibitors appeared an aliphatic amine group with a bulky lipophilic substituent at the end of the aliphatic chain. Regarding this characteristic, the compounds may be classified as amphiphiles [13].

The active and Na^+-dependent transport in the small intestinal epithelium depends on both the Na^+-sensitive carrier mechanism at the brush border membrane and the Na^+ gradient across this membrane maintained by a $(Na^+ + K^+)$-ATPase located at the basolateral membrane [14].

It is well known that drugs of similar structure like the amines used in this study inhibit $(Na^+ + K^+)$-ATPase activity in microsomal membranes [1]. The amines tested in this study, however, inhibited active transport in short-term incubations. This makes it rather likely that events at the brush-border membrane participate in the observed inhibition. Particularly, since agents acting at the basolateral membranes of the whole-tissue preparations often require a preincubation period to produce any inhibition [15]. In this connection, it is noteworthy that under the experimental conditions used in this study no significant effect of the amines on the intracellular Na^+ and K^+ concentrations could be observed. For harmaline which inhibits both the Na^+-dependent sugar carrier [5] and the $(Na^+ + K^+)$-ATPase [16], it was previously demonstrated that it can directly inhibit the Na^+-dependent active transport from the luminal side of the brush border and that the inhibition may not be secondary to its action on the ATPase [16,17].

The passive and the facilitated diffusion appeared to be not affected by the amines used in this study. As in analogy to the effects of biguanides [4], fructose absorption assumed to be cation independent but also carrier mediated was not impaired by the amines and therefore ionic interactions between amines and active transport systems may be suggested. The nature of these interactions, however, remains to be elucidated. Changes in the surface potentials of the brush-border membrane by the binding [1,18,19] of cationic amphiphiles to the membrane lipids as suggested for the action of biguanides [20] may contribute to the inhibition of the Na^+-dependent active transport. In this connection, it should be

mentioned that biguanides caused a noncompetitive inhibition of the active amino acid transport in the small intestine of hamsters [21]. Additionally the amines may compete with sodium ions for cation-binding sites at the carrier, a mechanism proposed for the action of harmaline [5,16,17] resulting in a pure competitive type of inhibition [22]. Furthermore, this kind of inhibition is obtained by omitting sodium ions from the mucosal bathing solution [10,23,24] and may also be observed by displacing sodium ions at the mucosal side, e.g. with polycations [8]. In fact, some of the present findings demonstrate the competitive character of the interaction between amines and sodium ions, at least with respect to the active sugar transport.

Connecting these considerations and the mode of inhibition of the active transport by the compounds studied in this investigation, it may tentatively proposed that the two mechanisms described above are reflected by the two groups of amines, the monoamines and the di- or triamines. (Despite the fact that the aromatic nitrogens in harmaline, pyrilamine, or imipramine are of low basicity, these amines which usually form only monohydrochlorides may be designated as di- or triamines.) According to the charge distribution in the amine molecule, the amines may preferentially interfere either with the transport-related Na^+-binding sites (K_m effect) or with the capacity of the translocation step (V_{max} effect).

Certainly these mechanisms are not the only ones by which lipid-soluble cations can impair small intestinal transport of sugars and amino acids. As suggested by the octanol-water partition coefficients and their correlation with the inhibition of the Na^+-dependent active transport, the lipid-solubility of the amines has to be considered for the elucidation of the mechanism. Particularly, since it was shown recently [25] that lipophilic substances modifying the fluidity of phospholipids in the brush border can inhibit non-specifically the D-glucose uptake into brush-border membrane vesicles. As shown by the reversibility experiment, a prolonged exposure of the tissue to the amines caused an increased irreversibility of the uptake inhibition. This might result from an increased influence of intracellular effects, e.g. inhibition of metabolic energy or/and inhibition of ($Na^+ + K^+$)-ATPase, due to the uptake of the organic bases as it was proposed for the action of chlorpromazine on the small intestinal uptake of methionine and galactose [26,27] and recently for the effect of phenformin on small intestinal sugar transport [28].

The organic amines may also interfere with other cation-dependent and absorption-related functions of the small intestine. This is indicated by the effect of some amines on the sucrase activity. Sucrase, an alkali metal ion activated, digestive enzyme [29,30] of the brush border is competitively inhibited by harmaline [3], an inhibitor thought to act as a specific Na^+-antagonist [17]. The amines investigated in this study may not this easily be classified as specific ion antagonists since they may even activate cation-dependent processes. The variability of the mode of action of the amines can be exemplified by pyrilamine which inhibits active transport competitively and activates sucrase, and by 3,3-diphenylpropylamine which is a noncompetitive inhibitor of the active transport and a competitive one of sucrase.

References

1 Nuhn, P., Frenzel,J. and Arnold, K. (1979) Pharmazie 34, 131–137
2 Dahlqvist, A.(1964) Anal. Biochem. 7, 18–23
3 Mahmood A. and Alvarado, F. (1977) Biochim. Biophys. Acta 483, 367–374
4 Caspary, W.F. and Creutzfeldt, W. (1971) Diabetologia 7, 379–385
5 Sepúlveda, F.V. and Robinson, J.W.L. (1974) Biochim. Biophys. Acta 373, 527–531
6 Caspary, W.F. and Creutzfeldt, W. (1972) Dtsch. Med. Wochenschr. 97, 394–396
7 Sundaresan, P.R. and Rivera-Calimlim, L. (1975) J. Pharmacol. Exp. Ther. 194, 593–602
8 Elsenhans, B., Blume, R., Lembcke, B. and Caspary, W.F. (1983) Biochim. Biophys. Acta 727, 135–143
9 Crane, R.K.and Mandelstam, P. (1960) Biochim. Biophys. Acta 45, 460–476
10 Alvarado, F. and Mahmood, A. (1974) Biochemistry 13, 2882–2890
11 Koopman, W. and Schultz, S.G. (1969) Biochim. Biophys. Acta 173, 338–340
12 Kessler, M., Acuto, O., Storelli, C., Murer, H., Müller, M. and Semenza, G. (1978) Biochim. Biophys. Acta 506, 136–154
13 Tanford, C. (1980) The Hydrophobic Effect, 2nd Edn., pp. 14–20, John Wiley and Sons, New York
14 Crane, R.K. (1968) in Handbook of Physiology (Code, C.F.,

ed.), Vol. III/6, pp. 1323–1351, American Physiological Society, Washington
15 Bihler, I. (1977) in Intestinal Permeation (Kramer, M. and Lauterbach, F., eds.), pp. 85–93, Excerpta Medica, Amsterdam
16 Sepúlveda, F.V., Buclon, M. and Robinson, J.W.L. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 295, 231–236
17 Alvarado, F., Brot-Laroche, E., L'Herminier, M., Murer, H. and Stange, G. (1979) Pflügers Arch.382, 35–41
18 Rooney,E.K., Gore, M.G. and Lee, A.G. (1979) Biochem. Pharmacol. 28, 2199–2205
19 Lüllmann, H. and Wehling, M. (1979) Biochem. Pharmacol. 28, 3409–3415
20 Schäfer, G. (1976) Biochem. Pharmacol. 25, 2005–2014
21 Caspary, W.F. and Creutzfeldt, W. (1973) Diabetologia 9, 6–12
22 Sepúlveda, F.V. and Robinson, J.W.L. (1978) J. Physiol. (Paris) 74, 585–590
23 Crane, R.K., Forstner, G. and Eichholz, A. (1965) Biochim. Biophys. Acta 109, 467–477
24 Curran, P.F., Schultz, S.G., Chez, R.A. and Fuisz, R.E. (1967) J. Gen. Physiol. 50, 1261–1286
25 Mitjavila, S., Fernandez, Y. and Boigegrain, R.A. (1983) Gastroenterol. Clin. Biol. 7, 508 (Abstr.)
26 Sundaresan, P.R. and Rivera-Calimlim, L. (1977) Biochem. Pharmacol. 26, 1411–1415
27 Sundaresan, P.R. and Rivera-Calimlim, L. (1978) Biochem. Pharmacol. 27, 2781–2786
28 Nicholls, T.J. and Leese, H.J. (1984) Biochem. Pharmacol. 33, 771–777
29 Alvarado, F. and Mahmood, A. (1979) J. Biol. Chem. 254, 9534–9541
30 Vasseur, M., Tellier, C. and Alvarado, F. (1982) Arch. Biochem. Biophys. 218, 263–274