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Active Transport Inhibition in Rat Small Intestine by Amphiphilic Amines: An *In Vitro* Study with Various Local Anaesthetics

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ABSTRACT. In the present investigation with rings of everted rat small intestine, amphiphilic amines such as local anaesthetics (e.g. lidocaine, procaine, tolycaine) were employed to study their effects on intestinal absorption of methyl α-D-glucoside, L-leucine, D-fructose, and 2-deoxy-D-glucose. All the amphiphilic amines tested, except for benzocaine, significantly inhibited Na^+-dependent active uptake of methyl α-D-glucoside and L-leucine while leaving uptake of D-fructose (facilitated diffusion) and 2-deoxy-D-glucose (passive diffusion) unaffected. Increasing concentrations of lidocaine in the incubation medium inhibited the uptake of methyl α-D-glucoside (IC_{50} ~3.5 mmol/L) and L-leucine (IC_{50} ~6 mmol/L) in a dose-dependent manner. Complete reversibility of the inhibitory effect could only be achieved at short-term incubations (≤2 min) and low lidocaine concentrations (≤3 mmol/L), otherwise inhibition became partially irreversible. Uptake kinetics of methyl α-D-glucoside and L-leucine in the presence of lidocaine revealed a significant increase in the apparent Michaelis constant, leaving the maximal transport capacity essentially unaltered. Reducing the Na^+ concentration in the incubation medium aggravated inhibition by lidocaine of the uptake of methyl α-D-glucoside. Analysis of the inhibition kinetics by Dixon plots revealed a competitive interaction between Na^+ and the amphiphiles. However, phlorizin binding was not affected by lidocaine. Changing the pH of the incubation medium from 5.6 to 8.0 increased the inhibitory effect of the amphiphiles, which indicated that the non-ionised and, thus, more lipophilic form participates in the mechanism of inhibition. However, benzocaine, a rather lipophilic local anaesthetic with no aliphatic amino group, did not impair active uptake of methyl α-D-glucoside. Whether the amphiphilic amines act by their partition into the membrane matrix or directly interact with sodium binding sites remains to be elucidated, however. BIOCHEM PHARMACOL **59**;8:907–913, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. amphiphilic cation; inhibition; intestinal absorption; local anaesthetics

Studying the effects of compounds interacting with membranes can contribute to the understanding of the mechanisms of solute translocation across biological membranes. Lipid-soluble, organic amines may be embedded into the lipid matrix of the membrane or bound to membrane phopholipids and membrane proteins, thus affecting membrane characteristics and functions [1, 2]. In their cationic form, they may also 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. Active transport of monosaccharides and amino acids by the brush-border carriers was found to be driven by co-transport of sodium ions. Therefore, it was conceivable that other cations interfere with these processes. In fact, various cationic organic compounds impair active transport in the small intestine. It was shown that active transport of monosaccharides and amino acids is impaired by compounds such as biguanides [3], harmaline [4], prenylamine [5], and chlorpromazine [6].

The results of studies employing polycations, which can be assumed to act only superficially at the apical membrane [7], suggested that cationic compounds may act directly with anionic residues at the luminal side of the mucosal surface and thus impair active transport. However, with smaller organic amines, lipophilic interactions between membrane and cationic agent are thought to be at least partially responsible for inhibiting sodium-dependent, small intestinal transport processes [8]. Since it was previously shown that antiarrhythmic and local anaesthetic drugs such as stirocainide [9] and lidocaine [10] can inhibit monosaccharide uptake in rat small intestine *in vitro* and since these drugs are known to impair entry of sodium ions into excitable cells, thus demonstrating some affinity towards membraneous sodium-binding ligands, local anaesthetics may provide a useful tool to further study the effects of amphiphilic cations on the absorptive functions of the

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small intestinal mucosa. Therefore, the present experiments are concerned with a characterisation of the effect of various local anaesthetics on rat small intestinal absorption *in vitro*. In particular, kinetic experiments and experiments concerning the pH dependency of the effect of the local anaesthetics may be expected to reveal more mechanistic features about the mode of action of these amphiphiles.

MATERIALS AND METHODS

Materials

2-[^{14}C (*U*)]Deoxy-D-glucose (11–13 GBq/mmol), D-[^{14}C (*U*)]fructose (7–13 GBq/mmol), L-[^{14}C (*U*)]leucine (>11 GBq/mmol), D-[1-^{3}H (*N*)]mannitol (1.7–2.0 TBq/mmol), D-[1-^{14}C (*N*)]mannitol (1.6–2.2 GBq/mmol), methyl α-D-[U-^{14}C]glucoside, and [phenyl-3,3′,5,5′-^{3}H, propanon-3-^{3}H]phlorizin (1.3–2.0 TBq/mmol) were purchased from NEN. The local anaesthetics articaine, butanilicaine, and tetracaine were gifts from Hoechst; lidocaine and etidocaine were gifts from Astra Chemicals. Also gifts were stirocainide, tolycaine, and benzocaine from Thiemann, Bayer, and Ritsert, respectively. Other local anaesthetics were purchased from Sigma Chemicals. All other chemicals were of analytical grade and obtained from standard commercial sources.

Animals

Female Sprague–Dawley rats (Süddeutsche Versuchstierfarm) weighing between 160 and 200 g were used throughout the study. The animals were housed in stainless steel cages on a 12-hr cycle of light and dark (dark 6 p.m. to 6 a.m.). Room temperature was kept at 22° and the humidity maintained at 50%. During an adaptation period of at least five days, the rats had free access to tap water and a standard rat diet (Alma H 1003, Botzenhardt); food was withdrawn 16 hr before starting the experiments.

Transport Experiments

The present experiments were performed by incubation of rings of everted rat small intestine *in vitro* according to well-established methods [11, 12]. Briefly, rings of everted small intestine (each approx. 0.5 cm in length) were prepared from 30-cm segments starting distal from the flexura duodenojejunalis (jejunum) and proximal from the valvula ileocoecalis (ileum). Usually, rings from four to five rats were pooled and randomised to minimise variability between animals. Eight to ten rings were incubated in a single 25-mL Erlenmeyer flask containing 5 mL of oxygen-saturated KH buffer.* If not otherwise stated, rings were incubated at 37° for 5 min in a medium with a pH of 6.8. This short-term incubation was chosen because within this period tissue uptake of solutes was linear both in controls and drug-containing solutions. Furthermore, this procedure minimises the influence of intracellular solute accumulation on the results, thus reflecting mainly initial uptake rates which were required for kinetic experiments. These were performed to establish the mode of inhibition of the local anaesthetics by determining their influence on the transport indices of methyl α-D-glucoside or leucine. The water-bath shaker operated at 120 cycles/min to minimise the effect of the unstirred water layer [13]. The buffers contained constant amounts of labeled transport substrate and labeled D-mannitol (either ^{3}H- or ^{14}C-labeled according to the label of the transport substrate) as tracers plus appropriate amounts of unlabeled transport substrate and D-mannitol (usually 1 mmol/L) to give the desired final concentrations. Phlorizin was used in analogous experiments, since this glucose transport inhibitor is only bound to but not transported by the glucose carrier. Binding of ^{3}H-labeled phlorizin was determined in 5-min incubations in oxygenated KH buffer (pH 6.8; 37°) using phlorizin instead of a transport substrate. Kinetic experiments were also performed to evaluate the effect of several local anaesthetics on the active transport of methyl α-D-glucoside at three different Na^{+} concentrations (35, 70, and 140 mmol/L). With a constant concentration of methyl α-D-glucoside (0.1 mmol/L), increasing concentrations of the drugs were employed. When these experiments were performed in buffer solutions of reduced Na^{+} concentrations (35 and 70 mmol/L), the corresponding amount of NaCl was isoosmotically replaced by adding mannitol.

Reversibility of the effect of the local anaesthetics was tested after a preincubation step in 5 mL oxygenated KH buffer (pH 6.8; 37°) with or without lidocaine (controls) added. Experiments were carried out by applying two different lidocaine concentrations (3 and 8 mmol/L) and two different preincubation periods (2 and 6 min). During preincubation, no transport substrate or mannitol was added and, thus, no uptake measured. After preincubation, everted rings were recovered by filtration and washed with 20 mL oxygenated KH buffer (pH 6.8; 37°) with or without lidocaine added in such a manner that three differently treated groups of everted rings were obtained, i.e. rings never treated with lidocaine (controls), rings treated with lidocaine only during preincubation (tissue-washed), and rings always treated with lidocaine (tissue-treated). Subsequently, tissue uptake of ^{14}C-labeled methyl α-D-glucoside (1 mmol/L) was determined (5-min incubations; KH buffer; pH 6.8; 37°) in the presence (tissue-treated) or absence (control; tissue-washed) of lidocaine.

Analytical Methods and Calculations of Results

At the end of the experiments, everted rings were recovered by filtration, blotted on gauze, weighed, placed in scintillation vials, and dissolved in 0.5 mL tetraethylammonium hydroxide (20% in water; Riedel de Haen). The procedure was sufficient to solubilise 250–350 mg of intestinal tissue

* *Abbreviations:* dGlc, 2-deoxy-D-glucose; Fru, D-fructose; KH buffer, Krebs–Henseleit phosphate buffer; Leu, L-leucine; Me-Glc, methyl α-D-glucoside; S_M, substrate concentration in the incubation medium; and S_T, substrate concentration in the intracellular fluid volume.

TABLE 1. Inhibition (percent of control) of small intestinal methyl α-D-glucoside and leucine uptake by various local anaesthetics

				Substrate			
				Methyl α-D-glucoside		L-Leucine	
Drug	Type	pK_a	P	Jejunum	Ileum	Jejunum	Ileum
Benzocaine	E	2.5	>400	96 ± 3	95 ± 8	—	—
Procainamide	A	9.2		68 ± 3*	46 ± 3*†	88 ± 10	73 ± 4*
Procaine	E	8.9	3	50 ± 3*	60 ± 11*	60 ± 3*	56 ± 5*
Stirocainide	O			70 ± 13	15 ± 5*†	74 ± 3*	42 ± 4*†
Lidocaine	A	7.9	36	36 ± 8*	37 ± 7*	50 ± 3*	37 ± 4*†
Articaine	A	7.8	17	40 ± 12*	8 ± 5*†	58 ± 5*	45 ± 4*
Tolycaine	A			28 ± 4*	20 ± 3*	57 ± 4*	46 ± 6*
Etidocaine	A	7.7	>400	40 ± 2*	35 ± 3*	—	—
Butacaine	E	9.0		53 ± 3*	25 ± 7*†	39 ± 5*	29 ± 3*
Butanilicaine	A		57	23 ± 4*	23 ± 5*	45 ± 4*	25 ± 3*†
Tetracaine	E	8.4	180	46 ± 8*	7 ± 4*†	20 ± 5*	12 ± 2*

Uptake of ^{14}C-labeled substrate into the tissue was determined *in vitro* with rings of everted rat small intestine. Substrate concentration, 1 mmol/L; drug concentration, 5 mmol/L; 5-min incubation (KH buffer, pH 6.8, 37°); — not determined. Type: A, amide; E, ester, O, oxime. P, partition coefficient (octanol/buffer, pH 7.4); means ± SEM (N = 5).

*Significantly different from the corresponding control.

†Significantly different from the corresponding inhibition in the jejunum ($P < 0.05$).

(8–10 rings) within 3–5 hr at 55° [14]. Thereafter, 15 mL of a scintillation cocktail (Omniscintisol, Merck) and 2 mL of water were added and the radioactivity measured in a scintillation spectrometer. Tissue accumulation or uptake was corrected for the mannitol space (extracellular fluid volume) and calculated as a distribution ratio S_T/S_M, i.e. the substrate concentration in the intracellular fluid volume (S_T) divided by the substrate concentration in the incubation medium (S_M), assuming an intracellular fluid volume of 80% of the tissue net weight [11, 12]. Tissue uptake rates, e.g. for the evaluation of transport kinetics, are expressed as substrate concentration in the intracellular fluid achieved per min (mmol/L/min). The unpaired Student's *t*-test ($P < 0.05$) was used to determine whether significant differences occurred in the indices measured.

RESULTS

All the various local anaesthetics–except benzocaine–inhibited the mucosal uptake of methyl α-D-glucoside and L-leucine, although their inhibitory effects differed greatly (Table 1). At a given substrate and inhibitor concentration, inhibition of methyl α-D-glucoside uptake ranged from 70 to 23% of control in the jejunum and from 60 to 7% of control in the ileum, while percentages for the inhibition of L-leucine uptake ranged from 88 to 20 and 74 to 12, respectively. On average, inhibition was slightly higher in the ileum than in the jejunum. When ranked according to their overall inhibitory effect, i.e. the average of the two substrates and segments as presented in Table 1, there was no systematic difference between the amides and esters tested. Furthermore, there was no clear ranking possible according to the pK_a values or the lipophilicity data, at least as far as data on physico-chemical properties that are currently available [15, 16].

To test the specificity of the inhibitory effect of the local anaesthetics on mucosal transport, different transport substrates were selected according to their mode of transport mechanism (Table 2). Active, sodium-dependent transport was strongly inhibited, as shown by the effect of tolycaine,

TABLE 2. Inhibition of small intestinal uptake of different substrates by various local anaesthetics

	Tissue uptake (S_T/S_M)					
Substrate	Control	+ Lidocaine	Control	+ Tolycaine	Control	+ Procaine
A						
Me-Glc	0.50 ± 0.08	0.13 ± 0.02*	0.70 ± 0.14	0.23 ± 0.05*	0.73 ± 0.16	0.30 ± 0.07*
Leu	0.96 ± 0.09	0.36 ± 0.04*	0.90 ± 0.07	0.54 ± 0.07*	0.95 ± 0.13	0.57 ± 0.11*
Fru	0.12 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.08 ± 0.02	0.08 ± 0.02
dGlc	0.10 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.17 ± 0.01	0.15 ± 0.01
B						
Me-Glc	0.68 ± 0.06	0.38 ± 0.02*	0.75 ± 0.12	0.11 ± 0.01*	0.92 ± 0.18	0.47 ± 0.08*
Leu	2.06 ± 0.27	0.70 ± 0.16*	2.20 ± 0.07	0.80 ± 0.04*	2.53 ± 0.23	1.32 ± 0.09*
Fru	0.09 ± 0.02	0.09 ± 0.02	0.17 ± 0.02	0.12 ± 0.02	0.14 ± 0.02	0.12 ± 0.03
dGlc	0.14 ± 0.01	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.21 ± 0.03	0.24 ± 0.01

Tissue uptake of ^{14}C-labeled substrates was determined in 5-min incubations in oxygenated KH buffer (pH 6.8; 37°) using rings of everted rat jejunum (A) and ileum (B); means ± SEM (N = 5). Drug concentration, 10 mmol/L; substrate concentration, 1 mmol/L; Me-Glc, methyl α-D-glucoside; Leu, L-leucine; Fru, D-fructose; dGlc, 2-deoxy-D-glucose.

*$P < 0.05$. Significance is relative to the corresponding control.

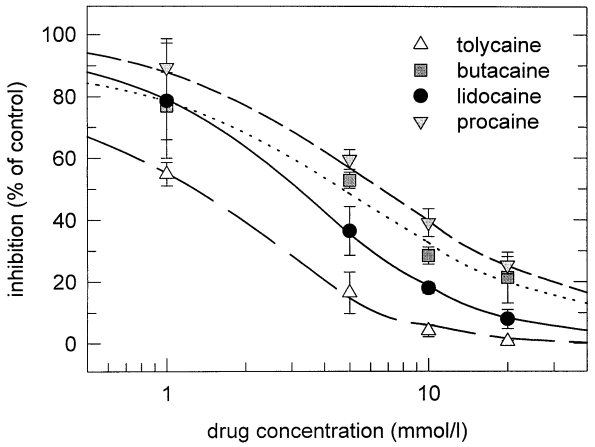

FIG. 1. Concentration-dependent inhibition of jejunal uptake of methyl α-D-glucoside (1 mmol/L) by various local anaesthetics. Sugar uptake was measured by 5-min incubations at 37° in oxygenated Krebs–Henseleit phosphate buffer (pH 6.8) with everted jejunal rings; sugar concentration: 1 mmol/L. Results are means of five separate experiments (±SEM).

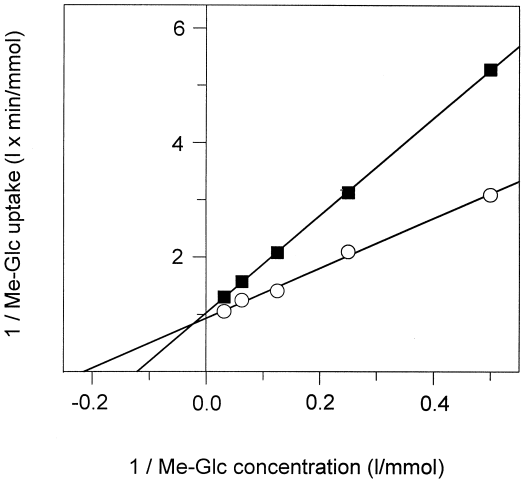

FIG. 2. Effect of lidocaine on the *in vitro* transport kinetics of methyl α-D-glucoside in rat jejunum. Tissue uptake rates were determined in the presence (■——■) or absence (○——○) of lidocaine (10 mmol/L) in the incubation medium. The 5-min incubations were carried out in oxygenated Krebs–Henseleit phosphate buffer (pH 6.8; 37°) using rings of everted rat jejunum. Results are means of five separate experiments (± SEM) and are plotted double reciprocally according to Lineweaver–Burk; Me-Glc, methyl α-D-glucoside.

lidocaine, and procaine on the mucosal uptake of methyl α-D-glucoside and L-leucine. In contrast, facilitated diffusion (Fru) and passive diffusion (dGlc) were not affected. Again, there was no difference between the ester or amide type of inhibitor. Applying increasing inhibitor concentrations (tolycaine, butacaine, lidocaine, and procaine, from 1 to 20 mmol/L) under the conditions described for the experiments above, the inhibition of the uptake of methyl α-D-glucoside was strictly dose-dependent (Fig. 1). The drug concentration needed to achieve 50% inhibition of the control uptake ranged from 1 to 7 mmol/L. A quite similar dose dependency was obtained for the four drugs when L-leucine was used as transport substrate (data not shown). The drug concentration for 50% inhibition of the control uptake of the amino acid (ranging from 4 to 9 mmol/L) was only slightly higher than that of the sugar. Further experiments of this type using other local anaesthetics were not performed, as the present experiments were thought to be representative of this group of drugs. Inhibition was found to be reversible to some extent (Table 3). Inhibition appeared to be completely reversible, in particular at low inhibitor concentrations (≤3 mmol/L) and short incubation periods (≤2 min). When the inhibitor concentration and incubation period increased, inhibition became partially irreversible.

The determination of the influence of lidocaine on transport indices was performed with methyl α-D-glucose and L-leucine as substrates. The results obtained and evaluated by Lineweaver–Burk plots (Figs. 2 and 3) show a drastic increase in the transport constants (K_T values) due to the addition of lidocaine to the incubation medium. When the V_{max} values were compared, however, no essential difference in the maximal transport capacities was observed. This competitive type of inhibition is shown for lidocaine and methyl α-D-glucoside uptake in jejunal rings (Fig. 2) and for lidocaine and L-leucine uptake in ileal rings

TABLE 3. Reversibility of the lidocaine-mediated inhibition of jejunal methyl α-D-glucoside uptake

Lidocaine concentration (mmol/L)	Preincubation period (min)	Preincubation medium: − Lidocaine	Preincubation medium: + Lidocaine			
		Control	Tissue-treated	% of control	Tissue-washed	% of control
3	2	0.42 ± 0.05	0.27 ± 0.05*	64 ± 12	0.50 ± 0.13	119 ± 31
3	6	0.46 ± 0.10	0.10 ± 0.01*	22 ± 2	0.19 ± 0.03*	41 ± 6
8	2	0.47 ± 0.09	0.08 ± 0.03*	17 ± 6	0.42 ± 0.10	89 ± 21
8	6	0.45 ± 0.05	0.04 ± 0.01*	9 ± 2	0.11 ± 0.02*	24 ± 4

Pretreatment of everted jejunal rings was carried out by preincubating the tissue in KH buffer (5 min; pH 6.8; 37°) without (− lidocaine) or with (+ lidocaine) lidocaine added. After preincubation, the tissue was washed with 20 mL oxygenated KH buffer with (tissue-treated) or without (control; tissue-washed) lidocaine added. Subsequently, tissue uptake of ^{14}C-labeled methyl α-D-glucoside (1 mmol/L) was determined (5-min incubations; KH buffer; pH 6.8; 37°) in the presence (tissue-treated) or absence (control; tissue-washed) of lidocaine. For further details, see Materials and Methods. Means ± SEM (N = 5).

*$P < 0.05$ (relative to the control).

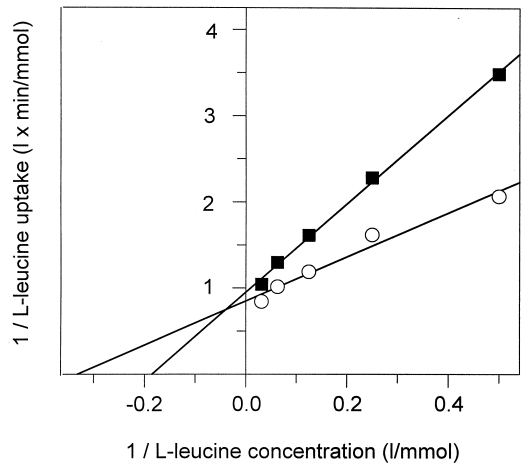

FIG. 3. Effect of lidocaine on the *in vitro* transport kinetics of L-leucine in rat ileum. The tissue uptake rates were determined in the presence (■——■) or absence (○——○) of lidocaine (10 mmol/L) in the incubation medium. Conditions were those depicted in the legend for Fig. 2; Results are presented as Lineweaver–Burk plot.

(Fig. 3), but was found in the other corresponding small intestinal segments as well (data not shown). Furthermore, analogous kinetic experiments performed using butacaine showed the same competitive type of inhibition for both substrates and both small intestinal segments (data not shown). Experiments were also performed at reduced sodium ion concentrations. The results obtained for lidocaine in jejunal rings (Fig. 4) are representative of its effect in ileal rings and of the effect of other drugs tested (procaine and tolycaine; data not shown). Applying the Dixon plot, the sodium ion is regarded as substrate for the process inhibited by the local anaesthetics. Thus, the common intercept between the three lines obtained for the effect of lidocaine at three sodium ion concentrations lying above the abscissa demonstrates a competitive type of inhibition between sodium ions and the drugs with respect to active monosaccharide uptake in the small intestine.

Besides solute uptake into the tissue, binding of phlorizin to the tissue was also tested in jejunal rings in the presence and absence of lidocaine or tolycaine (Table 4). For this purpose, phlorizin was used at concentrations in the range of its binding constant [17]. The effect of hydrolysis by phlorizin hydrolase on the results was minimised by short-term incubations [18]. Neither local anaesthetic had any effect on the amount of phlorizin bound to the tissue.

The inhibitory effect of the drugs was tested in incubation media of different pH. A range between pH 5.6 and 8 appeared reasonable to stay within physiological conditions. The results show that, when the pH was increased, methyl α-D-glucoside uptake decreased in the presence of the drugs (Fig. 5). The effect is clearly seen for lidocaine (Fig. 5A) and procaine (Fig. 5C), but is less distinct for etidocaine (Fig. 5B), which became less soluble in media with higher pH.

DISCUSSION

With the exception of benzocaine, all local anaesthetics tested in this study inhibited active sodium-dependent solute uptake. This is in accordance with previous findings where inhibition of sodium-dependent active transport of monosaccharides and amino acids in the small intestine required amphiphilic amines with an aliphatic rather than an aromatic amino group [8]. At a given substrate and drug concentration and at a fixed pH, however, it appears difficult to correlate one of the physico-chemical properties of the drugs with their inhibitory effect (see Results, Table 1). Owing to the short-term incubations, intracellular events by the various local anaesthetics, such as mitochondrial effects [19] or interactions with ATPases [20], can probably be excluded for the interpretation of the present results. Certainly, amphiphilic amines can be accumulated within cells, although this process likely needs more time than the present short-term incubations allowed. For an appreciable amount to accumulate, more than 10 min is likely required according to recent findings in human skin fibroblasts [21]. In fact, a reversible inhibition of sugar uptake was readily detected after 2-min incubations under the present conditions. As amide-type local anaesthetic drugs are exclusively metabolised in the liver and the ester-type compounds mainly by plasma pseudocholinesterases [22], effects by hydrolysis may also play no role.

From the present knowledge concerning the mode of action of the sodium–glucose co-transporter [23], it is assumed that the free carrier is negatively charged. Initial to the process of glucose translocation across the apical membrane, two sodium ions and one molecule of glucose are bound to the carrier. This sodium–glucose carrier complex, assumed to be neutral in charge, is then able to deliver

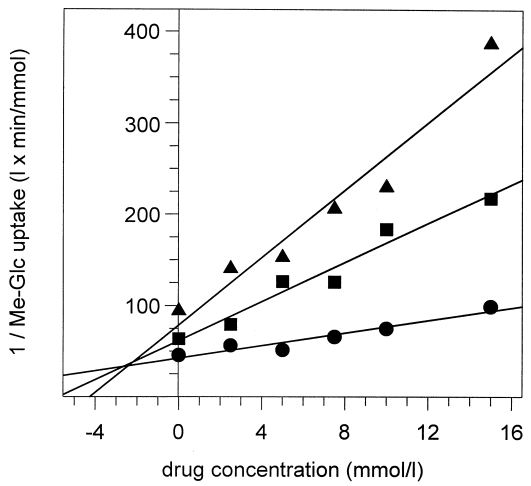

FIG. 4. Influence of three different Na^+ concentrations on the inhibitory effect of lidocaine as measured by the jejunal tissue uptake of methyl α-D-glucoside (Dixon plot). The 5-min incubations were carried out at sodium concentrations of 35 (▲——▲), 70 (■——■), and 140 mmol/L (●——●). The methyl α-D-glucoside concentration was 0.1 mmol/L and the results are means of five separate experiments; Me-Glc, methyl α-D-glucoside.

TABLE 4. Effect of local anaesthetics on jejunal phlorizin binding

Phlorizin (μmol/L)	Phlorizin bound (nmol/g tissue)			
	Control	+ Lidocaine	Control	+ Tolycaine
0.05	—	—	0.023 ± 0.003	0.019 ± 0.005
0.20	0.101 ± 0.003	0.102 ± 0.010	—	—
0.50	0.187 ± 0.012	0.230 ± 0.025	0.140 ± 0.024	0.149 ± 0.031
1.00	0.317 ± 0.013	0.402 ± 0.046	0.267 ± 0.029	0.258 ± 0.021

Binding of ^{3}H-labeled phlorizin was determined in 5-min incubations in oxygenated KH buffer (pH 6.8; 37°) using rings of everted rat jejunum; means ± SEM (N = 5); drug concentration, 10 mmol/L; — not determined.

glucose to the inside of the cell via a change in its conformation. There are several ways in which amphiphilic amines may interfere with this process. In principle, any amphiphile can interact with the lipid matrix of the cell membrane or with the membrane carrier protein. In any event, it is difficult to assess the contribution of each of these interactions to the overall process, particularly because lipid interactions can affect the function of a membrane protein indirectly [24, 25]. In addition, there is still the possibility that the amphiphiles react with both membrane constituents.

The present findings demonstrate that the sodium-dependent active transport systems for sugars and neutral amino acids are competitively inhibited by the presence of the amphiphiles. It is clear that the amphiphiles have no structural relationship to the monosaccharides and amino acids, so that the competitive nature may be derived from interactions between the cationic form of the amphiphilic amines and sodium ions. This view is supported by the results of the experiments in which the sodium ion concentration was reduced and the Dixon plots revealed a competitive nature between the amphiphiles and sodium ions. However, the results of the pH experiments seem to contradict this notion. From experimental findings and theoretical considerations, it is evident that compared to the protonated form the uncharged amines represent the more lipophilic form and therefore distribute into the lipid matrix of the membrane to a greater extent. According to the pK_a of the local anaesthetic, the ratio of the non-ionised base to the cationic form varies with the pH of the medium, so that the fraction of the non-ionised base increases by raising the pH. Increasing the pH of the incubation medium increased the inhibitory effect under the present conditions. Therefore, it is likely that the observed competitive nature between the amphiphilic amines and sodium is not solely a direct interaction between the corresponding cations. Thus, the present results demonstrate the existence of at least two different actions of the amphiphilic amines which probably co-exist and are not necessarily mutually exclusive.

The lack of a structural relationship between the amphiphilic amines and the substrates of the membrane carriers is not necessarily incompatible with the competitive nature of the inhibition, since membrane transport proteins can exhibit different binding sites for competitive inhibitors [26]. As binding of phlorizin is not impaired, it might be suggested that the amphiphilic amines impair the translocation step of glucose from the extracellular side into the cell, i.e. amphiphiles impair the conformational change of the sodium–glucose carrier complex necessary to release glucose into the cell.

The mode of action of the amphiphilic amines is certainly not clear yet; besides the pK_a in aqueous media and lipophilic properties, additional features of the amines might be involved. For instance, protein-binding properties may modify their effect. This view is derived from comparing the inhibitory effects of procaine with those of lidocaine at a pH of 8. Inhibition of methyl α-D-glucoside uptake by

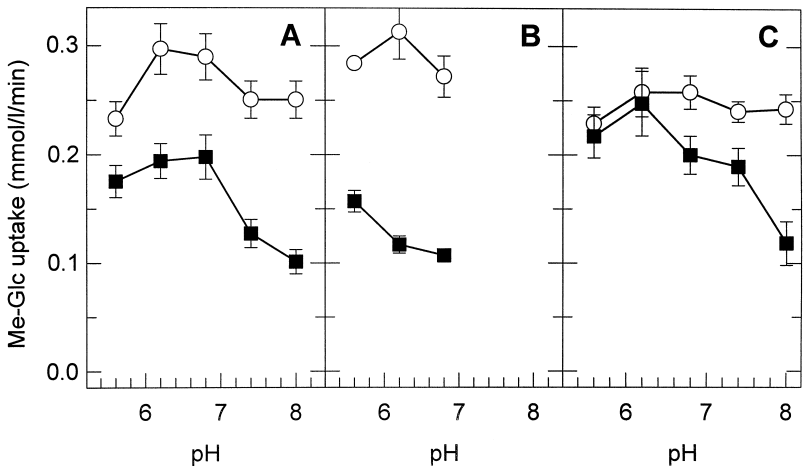

FIG. 5. pH dependence of the inhibitory effect of lidocaine (A), etidocaine (B), and procaine (C) on the tissue uptake of methyl α-D-glucoside (1 mmol/L). Uptake rates of methyl α-D-glucoside were determined in the presence (■——■) or absence (○——○) of the drugs (10 mmol/L) in the incubation medium. Owing to its reduced solubility at pH 7.4 and 8.0, the effect of etidocaine could not be determined at these pH values. Results are means of five separate experiments (± SEM); Me-Glc, methyl α-D-glucoside.

both local anaesthetics did not differ, although lidocaine, given its pK_a value and its partition coefficient, should have been embedded in the membrane to a much greater extent; protein binding of procaine can be considerably lower than that of lidocaine, however [15]. Binding to intestinal mucins in general or to sialic acid in particular may also play a yet undefined role in the interactions of amphiphilic amines with the apical membrane of enterocytes [27, 28]. Furthermore, one has to take into account that benzocaine did not impair sodium-dependent active transport and that all the other local anaesthetics employed had no effect upon uptake processes mediated by passive or even facilitated diffusion, i.e. sodium-independent transport processes. This strongly indicates a participation of the amino groups in the inhibition mechanism of sodium-dependent active transport. In this connection, it might be of interest that within the lipid matrix of the membrane, amphiphilic amines do not remain uncharged, but exist in an equilibrium with their protonated forms [2, 29].

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