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Effect of Loperamide on Na^+/D-Glucose Cotransporter Activity in Mouse Small Intestine

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Abstract

The μ-opioid agonist loperamide is an antidiarrhoeal drug which inhibits intestinal motility and secretion. Its anti-absorptive effects are less well investigated, but may be mediated through calmodulin. We have investigated further the effect of loperamide on the intestinal Na^+-dependent D-glucose transporter (SGLT1). Brush-border membrane vesicles were prepared from mouse small intestine, and uptake of [^{3}H]glucose was measured.

Na^+-dependent glucose uptake displayed the typical overshoot at 34 s; the peak value was 1·6 nmol mg^{-1}. The overshoot disappeared in the presence of phlorizin or when Na^+ was replaced by K^+. Extravesicular loperamide dose-dependently inhibited SGLT1 activity with an IC50 value of 450 μmol L^{-1}. Loperamide displayed a mixed inhibition type: the apparent V_{max} decreased from 0·9 to 0·5 nmol mg^{-1}/15 s, the apparent K_m increased from 0·23 to 1·13 mmol L^{-1} glucose. Na^+ kinetics were more complex, but loperamide inhibited net glucose uptake by 90% at 100 mmol L^{-1} Na^+. Glucose uptake was unchanged by agents affecting calmodulin activity. Loperamide inhibited intestinal Na^+, K^+-ATPase activity, whilst sucrase activity was unaffected.

SGLT1 activity was inhibited by loperamide, but this effect was not mediated through calmodulin. As this action is only evident at high concentrations of loperamide a non-specific mechanism may be involved.

Loperamide, a synthetic agonist at μ-opioid receptors, is widely used as an antidiarrhoeal agent. It is well established that the constipating action of this drug is due to its ability to inhibit both intestinal motility and net fluid secretion. Loperamide reduces intestinal motility and increases intestinal mean transit time (van Neuten et al 1974; Reynolds et al 1984; Chang et al 1986; Balkovetz et al 1987; Stoll et al 1987, 1988; Press et al 1991; Theodorou et al 1991). It also inhibits the secretory response of the intestine, blocking prostaglandin-induced fluid secretion by preventing the inhibition of electroneutral NaCl absorption (Hardcastle et al 1981).

Loperamide also inhibits intestinal absorptive processes (Chang et al 1986; Balkovetz et al 1987; Stoll et al 1987, 1988), and it has been shown to reduce the Na^+-dependent uptake of glucose and other nutrients in both intact rat small intestine and biopsies from human jejunum in-vitro (Hardcastle et al 1986, 1994). The effects of loperamide on intestinal absorptive processes have been reported to be mediated through an inhibition of calmodulin activity (Zavecz et al 1982; Diener et al 1988; Stoll et al 1988; Okhuysen et al 1995) and, in addition, an intracellular site of interaction of the drug with transporter proteins has been proposed (Stoll et al 1988). The anti-absorptive property of loperamide counters its antidiarrhoeal action and a better understanding of this effect may allow the development of more selective loperamide derivatives. Compounds lacking the ability to inhibit absorption would be more effective antidiarrhoeal agents, whilst compounds lacking the antidiarrhoeal properties may be of value in treating conditions of increased absorption such as the enhanced nutrient absorption in cystic fibrosis (Baxter et al 1990), which may contribute to the development of intestinal obstruction. We have undertaken further

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investigations into the influence of loperamide on intestinal nutrient absorption by examining its effects on the intestinal Na^+-dependent glucose transporter SGLT1 and the possible involvement of calmodulin using isolated brush-border membrane vesicles (BBMV) from mouse small intestine.

Materials and Methods

Animals

Adult male Swiss MF1 mice (20–25 g) were obtained from laboratory stock and were allowed free access to standard laboratory animal feed and water.

Materials

The hydrochloride salts of loperamide and the calmodulin antagonists *N*-(4-aminobutyl)-2-naphtalenesulphonamide (W-12) and *N*-(4-aminobutyl)-5-chloro-1-naphtalenesulphonamide (W-13) were from Sigma Chemical Co. (St Louis, MO). Phlorizin was from Phase Separations Ltd (Queensferry, UK). D-[2-^{3}H]Glucose, with a specific activity of 599–851 GBq $mmol^{-1}$, and the ECL Western blotting detection system were obtained from Amersham International PLC (Amersham, UK). Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) materials were from Bio-Rad (California). All other chemicals were analytical grade and obtained from commercial suppliers.

Preparation of brush-border membrane vesicles

Brush-border membrane vesicles (BBMV) were isolated from mouse ileum using a magnesium precipitation technique. Each preparation was derived from one mouse. Mice were killed by cervical dislocation and the proximal 25 cm of the small intestine, measured from the ligament of Treitz, was quickly removed. The ileal segment was flushed with ice-cold saline (150 mmol L^{-1} NaCl and 20 mmol L^{-1} HEPES/Tris at pH 7·4), cut open lengthwise and rinsed with the same saline. The mucosa was scraped off its supporting tissues with a microscope slide. Histological examination revealed that this method removed only villi from the mucosa. Scrapings were homogenized by 30 gentle strokes in a glass dounce homogenizer equipped with a tightly fitting Teflon pestle in 15 mL ice-cold solution containing 50 mmol L^{-1} mannitol and 20 mmol L^{-1} HEPES/Tris at pH 7·4. $MgCl_2$ was added to a final concentration of 10 mmol L^{-1}, and the homogenate was mildly agitated for 20 min. To collect the brush-border membranes, the homogenate was spun for 10 min in a cooled JA-20 rotor at 4725 g_{max} (6250 rev min^{-1}, *k*-factor = 7885) in a Beckman J2-MC centrifuge. The resulting supernatant was spun at 41 400 g_{max} (18 500 rev min^{-1}, *k*-factor = 900) for 30 min. The pellet was resuspended in 300 mmol L^{-1} mannitol, 0·1 mmol L^{-1} $MgSO_4$, 20 mmol L^{-1} HEPES/Tris (pH 7·4) by 25 passages through a 23-gauge needle. The suspension was spun again at 4725 g_{max} for 15 min, and the resulting supernatant again at 41 400 g_{max} for 30 min. The final pellet was resuspended in 200 mmol L^{-1} mannitol, 0·1 mmol L^{-1} $MgSO_4$, 20 mmol L^{-1} HEPES/Tris (pH 7·4).

Marker enzyme assays and vesicle orientation

The protein content of the BBMV preparation was determined with a commercial Coomassie Brilliant Blue reagent kit from Bio-Rad (München, Germany) using bovine serum albumin as a standard. Marker enzymes used to characterize the BBMV preparation were alkaline phosphatase (EC 3.1.3.1) and sucrase (EC 3.2.1.48) for brush-border membranes, $(Na^+ + K^+)$-ATPase (EC 3.6.1.3) for basolateral membranes, and succinate dehydrogenase (EC 1.3.99.1) for mitochondria. Assay procedures have been described elsewhere (Pennington 1961; Dahlqvist 1964; George & Kenny 1973; Mircheff & Wright 1976). Treatment with 0·4 mg mL^{-1} saponin unmasks latent alkaline phosphatase activity in membrane vesicles, and this fractional increase in enzyme activity was taken as an estimation of the proportion of inside-out oriented vesicles in the preparation.

SDS-PAGE and Western blotting

Samples from mucosal homogenates and BBMV were prepared by adding Laemmli electrophoresis sample buffer. After electrophoresis of 30 μg protein on a 8% polyacrylamide gel according to Laemmli (1970) the proteins were transferred to a nitro-cellulose membrane with a pore size of 0·45 μm. The blot was washed with phosphate-buffered saline (PBS) + 0·05% Tween (PBS-T). Non-specific binding sites were blocked with PBS-T to which 5% milk powder was added (60 min at room temperature). Incubations with the SGLT1 antibody 8821 and a horse radish peroxidase-conjugated rabbit secondary antibody were in PBS-T + 1% milk powder (60 min at room temperature). To visualize antibody binding the ECL Western blotting detection system was used. Blots were exposed to Hyperfilm-ECL.

Measurement of Na^+-dependent glucose uptake
Na^+-dependent glucose uptake in BBMV was measured using a rapid filtration protocol. BBMV were incubated at 20°C in a medium containing Na-gluconate and D-glucose as indicated in the legends, 740–1480 kBq D-[2-^{3}H]glucose mL^{-1}, 0·1 mmol L^{-1} $MgSO_4$, and 20 mmol L^{-1} HEPES/-Tris at pH 7·4. In control incubations, 0·25 mmol L^{-1} phlorizin was added, or Na-gluconate was replaced mole-for-mole by K-gluconate. In experiments where the substrate dependency of SGLT1 was investigated, Na-gluconate was replaced by K-gluconate to maintain the gluconate concentration and the combined concentration of Na and K at 100 mmol L^{-1}. Glucose was replaced by mannitol, and the combined monosaccharide concentration was maintained at 1 mmol L^{-1}. Loperamide was added from a 100 mmol L^{-1} stock in ethanol, controls received the ethanol vehicle only. The drug has a very poor solubility in saline at circumneutral pH; we were not able to dissolve loperamide at concentrations higher than 1 mmol L^{-1} in our assay buffer. The incubation was quenched by adding an 18-fold surplus of ice-cold stop buffer (165 mmol L^{-1} NaCl, 0·25 mmol L^{-1} phlorizin, and 10 mmol L^{-1} tris [hydroxy-methyl]-aminomethane (Tris) adjusted to pH 7·4 with HCl). The quenched medium was filtered immediately over a $-80\,kP_a$ vacuum using a cellulose nitrate membrane filter with a pore diameter of 0·45 μm (Whatman, Maidstone, UK) in a MilliPore filtration unit. The filter was immediately rinsed with 2 × 2 mL ice-cold stop buffer and transferred to a vial to which 4·5 mL liquid scintillation fluid (Emulsifier Safe from Packard, Meridan, USA) was added. Filters were allowed to dissolve, and analysed in a Packard Tri-carb 1600TR LSA. Results are expressed as nmol glucose mg^{-1}/15 s.

Analysis and statistics
Data were analysed using a non-linear regression data analysis program in which the Marquart algorithm was implemented (Leatherbarrow 1987). Standard errors of experimental data were used as explicit weighting factors. The time course of Na^+-dependent glucose uptake was fitted to the product of two exponential equations; one describing the active uptake of extravesicular glucose, and one describing the passive efflux of translocated intravesicular glucose:

$$J = \lfloor \text{Limit} \cdot (1 - e^{-k_1 \cdot t}) \rfloor \cdot e^{-k_2 \cdot t} \qquad (1)$$

where J represents Na^+-dependent glucose uptake. The rate constants for active glucose uptake and passive efflux are given by k_1 and k_2, respectively. The peak value of the overshoot was calculated by solving $dJ/dt = 0$, and substituting the obtained value for t in equation 1. Student's *t*-test for unpaired data and Welch's approximate (Ferguson 1971), and one-way analysis of variance, where appropriate, were used for statistical evaluation. Data are presented as mean value ± s.e.m., with the number of preparations (n) in parentheses.

Results

BBMV characteristics
Biochemical characteristics of our BBMV preparation are shown in Table 1. The membrane preparation was enriched 8-fold for the brush-border membrane markers alkaline phosphatase and sucrase. Figure 1 shows that BBMV were enriched in SGLT1 immunoreactivity with respect to the homogenates from which the preparations were derived. Contamination with other membrane fractions was low, as indicated by the enrichment factors < 1 for Na^+, K^+-ATPase and succinate dehydrogenase. Unmasking of latent alkaline phosphatase activity by saponin treatment revealed 6·5 ± 2·1% (n = 9) inside-out oriented vesicles.

Na^+-dependent glucose uptake
Figure 2A shows the time course of glucose uptake in the presence of an inwardly directed 100 mmol L^{-1} Na^+ gradient. Standard errors represent mainly interassay variation. An overshoot is observed after which the glucose content of the BBMV falls to an equilibrium value. The overshoot disappeared in the presence of 0·25 mmol L^{-1} phlorizin or when Na^+ was replaced by equimolar K^+. Under all incubation conditions, equilibrium values at t = 15 min were similar. We obtained net Na^+-dependent glucose uptake by subtracting passive uptake measured in the presence of a K^+ gradient from active uptake in the presence of a Na^+ gradient (Figure 2B). The data points are well described by equation 1. The value of the overshoot was calculated to be 1·6 nmol mg^{-1} at t = 34 s. The rate constants for active glucose uptake and passive efflux were calculated to be 5·0 min^{-1} and 0·3 min^{-1}, respectively; the passive efflux rate thus being 17-times slower than the active Na^+ gradient-driven uptake. The time course was linear up to 20 s. We chose a time point of t = 15 s to measure unidirectional uptake rates.

Effect of loperamide on glucose uptake

Dose-dependent inhibition. Loperamide dose-dependently inhibited Na^+-dependent unidirec-

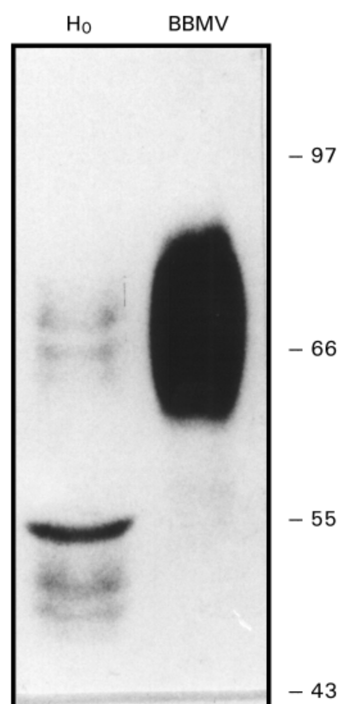

Figure 1. Immunoblot analysis of SGLT1 protein (molecular mass 74 kDa) in BBMV and intestinal mucosal homogenates (H_0) from mouse intestine, using the anti-SGLT1 8821 antibody. The preparations were normalized for total protein. Molecular weights (kDa) are indicated on the right.

tional glucose uptake in BBMV with a calculated IC50 of 450 μmol L^{-1} (Figure 3). Attempts were made to load vesicles with loperamide using the pre-incubation method described by Stoll et al (1988). However, we observed a 32% inhibition of Na^+-dependent glucose uptake caused by pre-incubation with the ethanol vehicle alone, with no additional effects of loperamide. Passive uptake in the presence of a K^+ gradient was unaffected. This led us to conclude that any effects observed with intravesicular loperamide can be attributed to the ethanol vehicle.

Substrate kinetics. Figure 4B shows that data points converge well on a linear Eadie-Hofstee plot, and we conclude that unidirectional Na^+ gradient-driven glucose uptake can be described adequately by simple Michaelis-Menten kinetics with respect to the substrate D-glucose (Figure 4A). In the presence of 450 μmol L^{-1} loperamide, the apparent limiting transport rate (V_{max}) decreased from 0·9 to 0·5 nmol mg^{-1}/15 s, and the apparent Michaelis-Menten constant (K_m) increased from 0·23 to 1·13 mmol L^{-1} glucose. The apparent K_m in control BBMV is similar to K_m values reported for rabbit and rat SGLT1 (Steel & Hediger 1998). The decreases in apparent V_{max}

Table 1. Enrichment and relative recoveries of marker enzyme activities in mouse ileal brush-border membrane vesicle preparations.

Marker	Enrichment[a]	Recovery[b] (%)
Alkaline phosphatase	7·8 ± 0·6 (10)	10·2 ± 1·7 (8)
Sucrase	8·0 ± 1·2 (10)	9·8 ± 2·4 (8)
Na^+, K^+-ATPase	0·9 ± 0·3 (9)	1·4 ± 0·6 (7)
Succinate dehydrogenase	0·6 ± 0·2 (9)	0·6 ± 0·1 (7)
Protein	–	1·3 ± 0·2 (8)

[a]Enrichment is the ratio of the specific activities in the BBMV preparation and the initial homogenate. [b]Recovery is calculated as the percentage of the total activity (equals specific activity × total protein content) in the BBMV preparation relative to that in the initial homogenate.

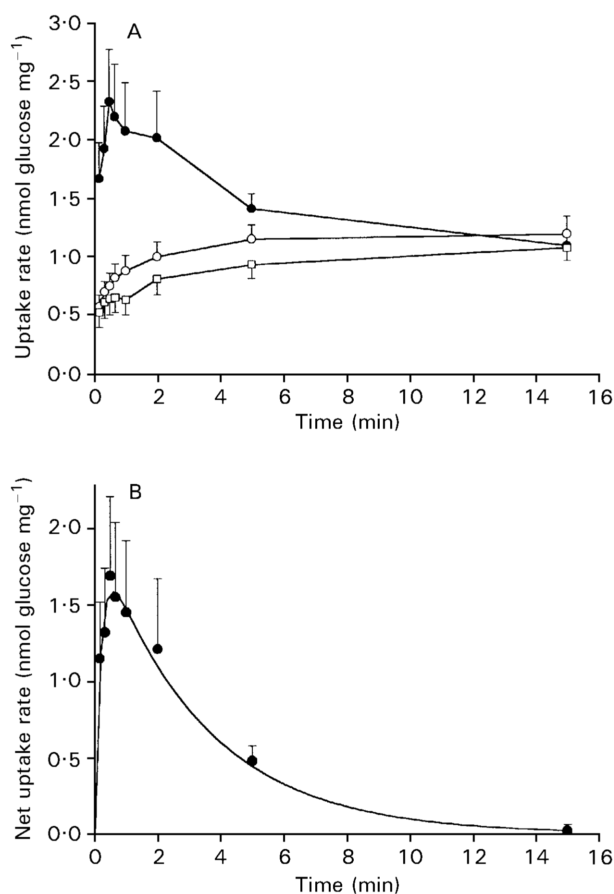

Figure 2. A. Glucose uptake in mouse ileal BBMV (n = 5). The intravesicular medium contained (mmol L^{-1}) 200 mannitol, 0·1 $MgSO_4$, and 20 HEPES/Tris (pH 7·4), the extravesicular medium contained (mmol L^{-1}) 1·0 D-glucose, 0·1 $MgSO_4$, 20 HEPES/Tris (pH 7·4) and either 100 mmol L^{-1} Na-gluconate (●), 100 mmol L^{-1} Na-gluconate + 0·25 mmol L^{-1} phlorizin (○), or 100 mmol L^{-1} K-gluconate (□). B. Net Na^+ gradient-dependent glucose uptake. Data points were fitted to equation 1, parameters were calculated to be: limit = 2 nmol mg^{-1}, k_1 = 5·0 min^{-1}, k_2 = 0·3 min^{-1}. The peak value of the overshoot was calculated to be 1·6 nmol mg^{-1} at t = 34 s.

and in the ratio V_{max}/K_m are characteristic for a mixed inhibition type (Cornish-Bowden 1995).

Active glucose uptake measured as a function of extravesicular Na^+ was inhibited by up to 60% at 10 mmol L^{-1} Na^+, and up to 90% at 50 and 100 mmol L^{-1} Na^+ by 450 μmol L^{-1} loperamide (Figure 5A). We could only resolve unreliable values for kinetic parameters (errors > 30%) because glucose uptake was inhibited to such a high extent, and because control uptake values did not saturate at Na^+ concentrations $\leq$100 mmol L^{-1}. When *N*-methyl-D-glucamine-gluconate was used to maintain the combined Na^+ and *N*-methyl-D-glucamine concentration at 100 mmol L^{-1}, a similar time course as with K-gluconate was observed (results not shown). A lower loperamide concentration of 200 μmol L^{-1} did not inhibit active glucose uptake; extending the Na^+ concentration range to 500 mmol L^{-1} did not saturate active glucose uptake (Figure 5B). Therefore, we were not able to resolve reliable saturation kinetics of active glucose uptake and the type of inhibition by loperamide with respect to the substrate sodium.

Calmodulin reagents and glucose uptake

Loperamide has been suggested to act on intestinal transport processes by inhibiting calmodulin activity (Zavecz et al 1982; Stoll et al 1988). For this mechanism to explain the inhibition of active glucose uptake by loperamide, SGLT1 must be sensitive to calmodulin reagents. In control experiments net glucose uptake was 0·9 ± 0·4 nmol mg^{-1}/15 s (n = 6) and this was unaffected by ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) at

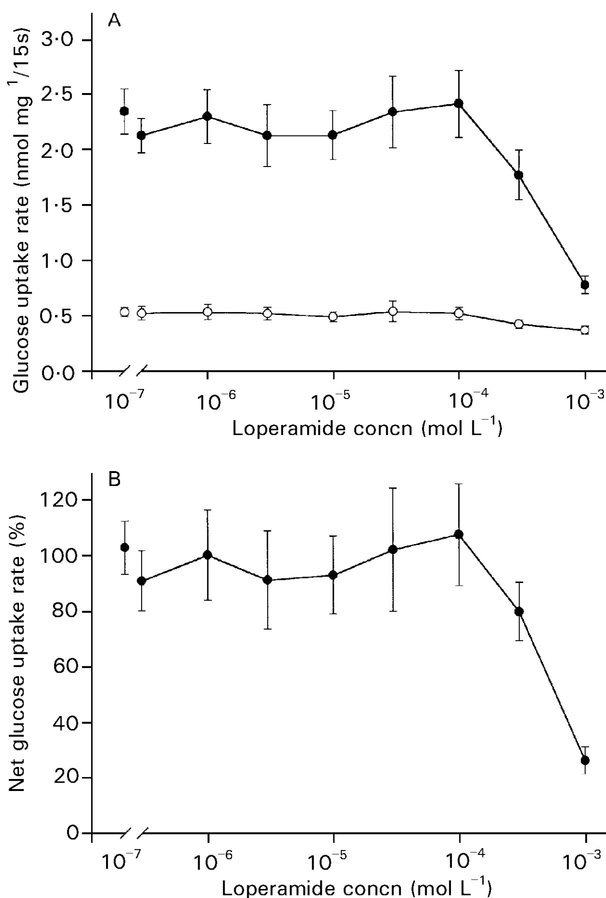

Figure 3. A. Dose-dependent inhibition of glucose uptake in mouse ileal BBMV by loperamide (n = 6). The intravesicular medium contained (mmol L^{-1}) 200 mannitol, 0·1 $MgSO_4$, and 20 HEPES/Tris (pH 7·4), the extravesicular medium contained (mmol L^{-1}) 1·0 glucose, 0·1 $MgSO_4$, 20 HEPES/Tris (pH 7·4) and either 100 mmol L^{-1} Na-gluconate (●) or 100 mmol L^{-1} K-gluconate (○). B. Dose-dependent inhibition of net Na^+-dependent glucose uptake by loperamide. Uptake at 100% equals 1·8 nmol mg^{-1}/15 s, IC50 = 450 μmol L^{-1} loperamide.

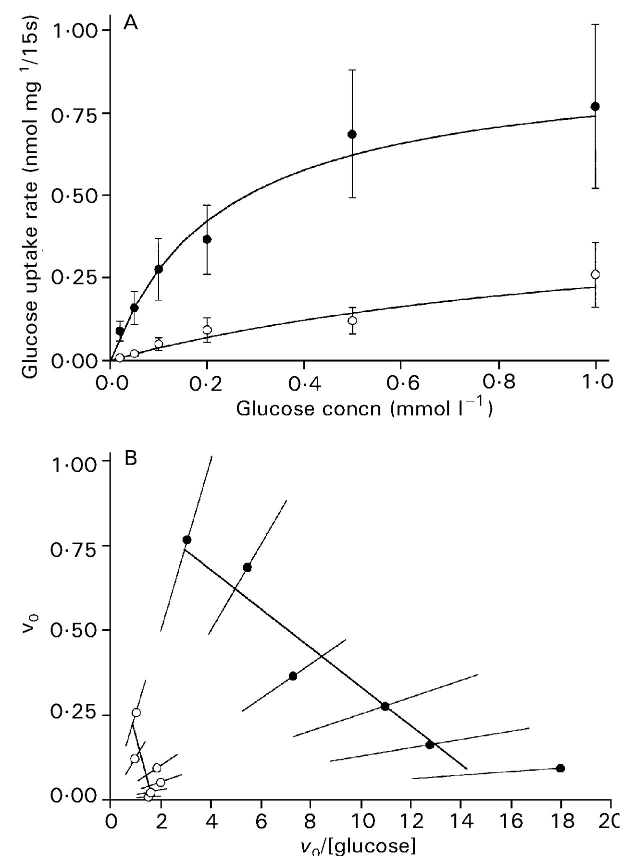

Figure 4. A. Net Na^+-dependent glucose uptake in mouse ileal BBMV as a function of the extravesicular glucose concentration, in the presence of 450 μmol L^{-1} loperamide (○) or vehicle only (●) (n = 7). Data were fitted to a simple Michaelis-Menten equation. B. Eadie-Hofstee transformation of the data. Calculations of kinetic parameters are: apparent K_m = 0·2 mmol L^{-1} glucose, apparent V_{max} = 0·9 nmol mg^{-1}/15 s, and apparent K_m = 1·1 mmol L^{-1} glucose, apparent V_{max} = 0·5 nmol mg^{-1}/15 s for uptake in the presence of vehicle (●) and 450 μmol L^{-1} loperamide (○), respectively.

0·1 mmol L^{-1}, 0·1 mmol L^{-1} $CaCl_2$, or 1–100 μmol L^{-1} of W-12 and W-13, an analogue pair of low- and high-affinity calmodulin antagonists, respectively (n = 6, $P = 0·997$, results not shown).

Effect of loperamide on sucrase and Na^+, K^+-ATPase activities

The activity of the sodium-translocating pump Na^+, K^+-ATPase, an integral membrane protein, was inhibited by 51% in the presence of 450 μmol L^{-1} loperamide. In contrast, the activity of sucrase, an extracellular enzyme projecting from the plasma membrane into the gut lumen, was unaffected (Table 2).

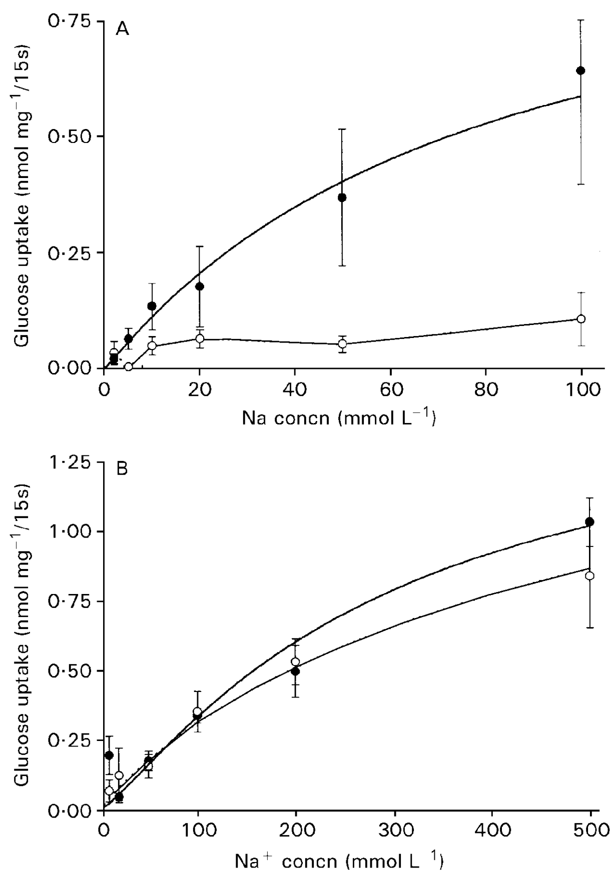

Figure 5. Net Na^+-dependent glucose uptake in mouse ileal BBMV as a function of the extravesicular Na^+ concentration. A. Uptake in the presence of 450 μmol·L^{-1} loperamide (○) or vehicle only (●) (n = 5). B. Uptake in the presence of 200 μmol·L^{-1} loperamide (○) or vehicle only (●) (n = 5). Note the different concentration scales on the abscissas.

Discussion

We conclude from this study that loperamide inhibited SGLT1 activity in the apical membrane of mouse small intestinal enterocytes. However, in comparison with its antidiarrhoeal action, the inhibition of glucose uptake by loperamide occurred at relatively high concentrations; we calculated an IC50 value of 450 μmol L^{-1}. This IC50 value is in the same range as that inhibiting Na^+-dependent amino acid absorption in intact rat intestine (Hardcastle et al 1986). Stoll et al (1988) were unable to demonstrate an inhibitory effect of loperamide on Na^+-dependent glucose transport in human ileal BBMV. This was probably due to the low loperamide concentration (10 μmol L^{-1}) used in their study.

It is unlikely that an interaction with opioid receptors could explain the inhibitory action of loperamide on Na^+-dependent glucose uptake. In intact intestine the anti-absorptive effect is neither mimicked by morphine nor antagonized by naloxone (Hardcastle et al 1986). Moreover, there is no evidence for the presence of opioid receptors on isolated enterocytes or brush-border membrane fractions (Gaginella et al 1983; Binder et al 1984; Balkovetz et al 1987; Schulzke et al 1990). It is possible that loperamide interacts directly with the SGLT1 protein, but this effect is probably not specific as it has been shown that loperamide also inhibits several other intestinal transporters as well. For example the IC50 values for inhibition of a Na^+/H^+ exchanger in rabbit small intestinal BBMV (Balkovetz et al 1987), glycine and galactose transport in rat small intestinal everted sacs (Hardcastle et al 1986), and Na^+/glucose co-

Table 2. Effect of 450 μmol L^{-1} loperamide on the activities of $Na^+ + K^+$-ATPase in intestinal mucosal homogenates and sucrase in purified BBMV from mouse ileum.

Treatment	Na^+, K^+-ATPase activity (μmol P_i h^{-1} mg^{-1})	Sucrase activity (μmol glucose h^{-1} mg^{-1})
Vehicle (0·45% ethanol)	5·10 ± 0·85	105·7 ± 15·1
Loperamide (450 μmol L^{-1})	2·50 ± 0·55	104·4 ± 16·4

Results are from four different preparations, control incubations contained 0·45% ethanol vehicle only. 95% CI for sucrase activity: control 52·4–156·4, loperamide 57·8–153·6 μmol glucose h^{-1} mg^{-1} ($P = 0·96$); for Na^+, K^+-ATPase activity: control 2·4–7·8, loperamide 0·7–4·3 ($P = 0·04$). P_i is inorganic phosphate.

transport and Na^+, K^+-ATPase in mouse ileal BBMV (this study) are at least one order of magnitude higher than the effective concentrations of loperamide at μ-opioid receptors and Ca^{2+} channels (Reynolds et al 1984; Chang et al 1986; Kromer 1995), which are in the range of 0·1–10 μmol L^{-1}. This low-affinity, non-specific inhibitory effect of loperamide on different transporters could result from an interaction of the drug with some common cellular regulator. Indeed, results from several studies suggest that there is a calmodulin-mediated component in the antidiarrhoeal action of loperamide (Merritt et al 1982; Zavecz et al 1982; Diener et al 1988; Okhuysen et al 1995). Results from radioimmunoassays on isolated BBMV from human jejunum, and immunocytochemistry on intact Necturus intestinal mucosa indicated that there was a considerable calmodulin fraction bound to the brush-border membrane (Stoll et al 1987, 1988; Scully et al 1988). However, Na^+-dependent glucose uptake by membrane vesicles is insensitive to reagents that are known to affect calmodulin activity, indicating that SGLT1 in our BBMV preparation is not sensitive to calmodulin, and thus excluding calmodulin as a mediator of the inhibitory action of loperamide. Our observation of the inhibition of Na^+, K^+-ATPase, but not sucrase, by loperamide could indicate a change in biophysical membrane properties induced by loperamide, affecting the activities of integral membrane proteins involved in transmembrane translocation of substrates. Brush-border membrane structure is known to affect protein activity (Proulx 1991), e.g. it has been shown that changes in brush-border membrane fluidity correlate well with Mg^{2+}-ATPase activity (Kitagawa et al 1995). We suggest that the low-affinity, non-specific inhibitory effect of loperamide on SGLT1 and Na^+, K^+-ATPase could be due to an induced change in membrane fluidity.

With respect to the substrate glucose, we found the inhibition by loperamide to be of a mixed type. This observation is corroborated by results from Balkovetz et al (1987) who, in a study on the human placental Na^+/H^+ exchanger, also concluded that loperamide inhibition was of a mixed type. A mechanism of non-specific inhibition of membrane transporter proteins by loperamide through changes in membrane fluidity is compatible with this mixed inhibition type.

In conclusion, our data shows that luminal loperamide inhibits the activity of the intestinal Na^+/D-glucose cotransporter, a property that will contribute to its anti-absorptive effect in mammalian intestine. We do not know of data pertaining to intestinal luminal loperamide concentrations after oral administration. However, it has been reported that in-vitro transepithelial intestinal transport of loperamide is poor, and that the drug is likely to accumulate in the intestinal mucosa (Miyazaki et al 1982; Brayden et al 1997). Thus, it could be possible that an oral intake of 4 mg (8 μmol) loperamide, a recommended initial dose to treat traveller's diarrhoea, or higher doses to treat short bowel syndromes may generate sufficiently high luminal concentrations to inhibit absorptive processes. However, the observed high concentrations required to produce an inhibition and the non-specific nature of this effect make the drug an unlikely candidate for the development of a treatment for intestinal hyperabsorption. Investigations into the action of loperamide analogues may reveal a compound that retains the antidiarrhoeal actions and thus has even greater antidiarrhoeal efficacy.

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