Na^+-coupled D-glucose uptake and membrane order of enterocyte brush border membrane vesicles, under the effect of a series of *N*-phenylcarbamates

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The importance of the hydrophobic effect of exogenous substances and of modifications of membrane order on D-glucose uptake are still poorly defined. Our results show that the concentrative Na^+-coupled D-glucose uptake of rat enterocyte brush border membrane vesicles is inhibited by *N*-phenylcarbamate derivatives. Unlike other series of lipophilic compounds inhibiting D-glucose transport, these *N*-phenylcarbamates increase the membrane order. However, since the concentrations required for membrane order increase are much greater than those active on D-glucose uptake, the effects on lipid order cannot be responsible for the inhibition of D-glucose uptake. Measurements of D-glucose uptake under conditions of Na^+ equilibrium show that these carbamates do not act directly on the carrier but indirectly by favouring the dissipation of the Na^+ gradient.

Glucose transport *Membrane fluidity* *ESR* N-*Phenylcarbamate* *Hydrophobic effect* *(Rat intestinal brush border)*

1. INTRODUCTION

Several substances are known to have an inhibiting effect on Na^+-coupled D-glucose uptake of enterocyte brush border membrane vesicles. Two main mechanisms are proposed to explain this effect: inhibition of the carrier activity as observed with phlorizin [1–3] and dissipation of the Na^+ gradient due to an increase in membrane permeability as observed with -SH reagents [4,5] or compounds disturbing hydrophobic interactions such as ethanol [6] and other aliphatic alcohols [7]. In the latter case, the decrease in glucose transport can be correlated with an increase in membrane disorder.

In analysing the role of membrane order we studied the effect of other lipophilic substances on D-glucose uptake, and especially that of substances which could induce an increase in membrane order. Fluorescence polarization measurements [8] have shown that chlorpropham (*N*-phenylcarbamate) decreases the fluidity of plant mitochondrial membrane. Both chlorpropham and propham are used as herbicides and can be considered as potential food contaminators. On the basis of their hydrophobic interactions and when compared to *n*-aliphatic alcohols, the high partition coefficient of these compounds indicates an inhibiting activity at a fairly low concentration. Encouraging preliminary results led us to synthesize other derivatives in this series, in order to check the relationship between the inhibition of D-glucose uptake and the lipophilic character of the inhibitors. The results obtained were compared to the modifications of the membrane order measured by ESR.

2. MATERIALS AND METHODS

2.1. *Brush border membrane vesicles (BBMV)*

BBMV were prepared from rat small intestine [9,10]. After protein determination [11] BBMV

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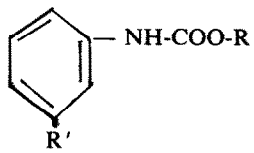
were suspended in 300 mM mannitol, 2 mM Tris-HCl, pH 7.5 (20 mg protein/ml), for ESR studies or in 300 mM mannitol, 10 mM Hepes-Tris, pH 7.5 (2–4 mg protein/ml), for D-glucose uptake studies. D-Glucose uptake by BBMV was determined by a filtration technique [12]. The measurements were made in the presence of a Na^+ gradient as in [7]; details are given in fig.1. D-Glucose uptake was also studied under conditions of Na^+ equilibrium to differentiate the direct effect of *N*-phenylcarbamates on the carrier from the indirect action due to dissipation of the Na^+ gradient (see fig.3). For ESR studies, spin label (*N*-oxyl-4′,4′-dimethyloxazolidine derivative of 12-ketostearic acid) was incorporated into BBMV in a spin label/lipid molar ratio of about 1/100. ESR spectra were recorded with a Brucker ER 200 DX band spectrometer (at 25°C). $2T'_{\parallel}$ was taken as a measure of probe ordering related to the amplitude of anisotropic motion of the label in the membrane [13].

2.2. *Chemicals*

The compounds used (table 1) belong to the *N*-phenylcarbamate series. Derivatives 1 (propham) and 3 (chlorpropham) were purchased from Ets Cluzeau (33220 Sainte-Foy-la-Grande, France) (purity: 99%). Derivatives 2 and 4 were synthesized [15] and their purity, checked by TLC [16], was over 99%. An ethanol solution of these compounds was used, the concentration of ethanol in the reaction medium (1%) had no effect on the D-glucose uptake or on the value of parameter $2T'_{\parallel}$ [7].

Table 1

Structure and partition coefficient of *N*-phenylcarbamates



Compound	R	R′	log*P* (octanol/water)
1	$-CH(CH_3)_2$	H	2.27[a]
2	$-CH_3$	Cl	2.71[b]
3	$-CH(CH_3)_2$	Cl	3.51[a]
4	$-C(CH_3)_3$	Cl	3.87[b]

[a] Values communicated by T. Fujita (unpublished)
[b] Values calculated by reference to compound 3, according to Hansch and Deutsch [14]

3. RESULTS

3.1. *Effects of* N-*phenylcarbamates on D-glucose uptake in the presence of a* Na^+ *gradient*

Fig.1 shows the evolution of D-glucose uptake and D-mannose uptake in the presence or absence of 0.5 mM chlorpropham during a 0–90 min incubation. In the absence of carbamate, there was a typical transient Na^+-dependent accumulation of D-glucose which reached a maximum between 15 and 20 s: an equilibrium level was reached after 90 min. 0.5 mM chlorpropham inhibited the tran-

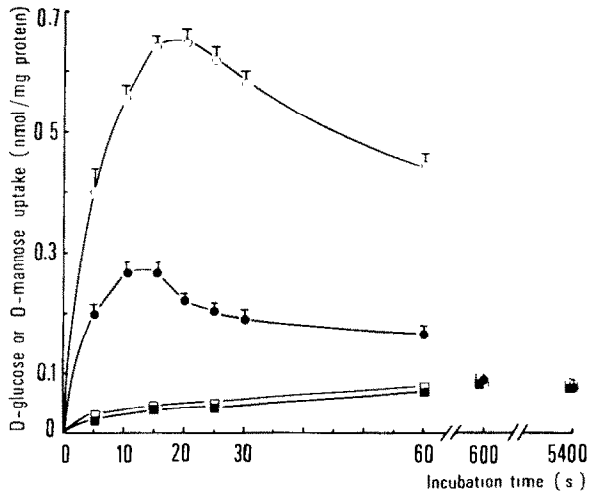


Fig.1. Time course of D-glucose uptake (○, controls; ●, 0.5 mM chlorpropham) and D-mannose uptake (□, controls; ■, 0.5 mM chlorpropham) into BBMV at 25°C in the presence of an Na^+ gradient. Data represent the means of 4 different preparations. SE bars are indicated except when SE was less than the size of the symbol. 50 μl of BBMV were preincubated for 10 min with or without chlorpropham. Uptake was initiated by adding 50 μl of 0.2 mM D-[^{3}H]glucose, 0.2 mM D-[^{14}C]mannose and 200 mM NaSCN contained in mannitol Hepes-Tris buffer, with or without chlorpropham. Incubation was stopped by addition of 3 ml of an ice-cold stop solution (250 mM NaCl, 1 mM Tris-HCl, pH 7.5) and filtration through Millipore filter HATF (pore size: 0.45 μm). Filters were washed twice with 3 ml of stop solution.

sient accumulation of D-glucose, but at 90 min D-glucose uptake was identical to that of the controls. The difference between D-glucose uptake and D-mannose uptake represents the net D-glucose uptake. Fig.2 shows the percentage of net D-glucose uptake, compared with the control (at 15 s incubation), against carbamate concentration. A linear relationship for each carbamate can be seen between 0.1 and 2 mM. From the results shown in fig.2, the carbamate concentrations (C) producing 50% inhibition of D-glucose uptake were calculated. They were found to be 0.98 mM for 1, 0.55 mM for 2, 0.24 mM for 3 and 0.21 mM for 4. The linear relationship between log $1/C$ and log P [17] is: log $1/C$ = 0.426 log P – 0.926 (r = 0.988).

3.2. *Effects of* N-*phenylcarbamates on D-glucose uptake under conditions of* Na^+ *equilibrium*

Fig.3 shows that in the presence of Na^+ but with no electrochemical Na^+ gradient, 1 mM chlorpropham does not modify D-glucose uptake. Na^+-independent D-glucose uptake was not modified either (measurements made in the presence of KCl). Similar results were obtained with the 3 other N-phenylcarbamates at the same concentration.

3.3. *Effects of* N-*phenylcarbamates on the lipid bilayer order*

The mean ± SE (n = 4) of parameter $2T'_{\parallel}$ was 44.3 ± 0.13 G for the BBMV controls. Incubation

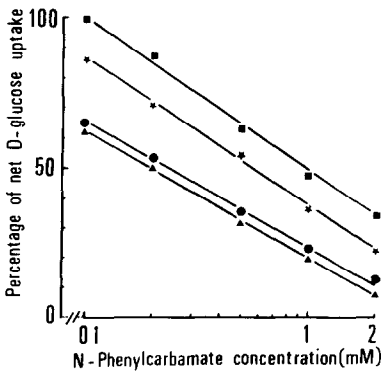


Fig.2. Effects of N-phenylcarbamates on net D-glucose uptake (15 s) in the presence of an Na^+ gradient. The final concentrations are indicated on the abscissa for compounds (table 1): 1 (■), 2 (★), 3 (●) and 4 (▲). Data represent means for 4 different vesicle preparations.

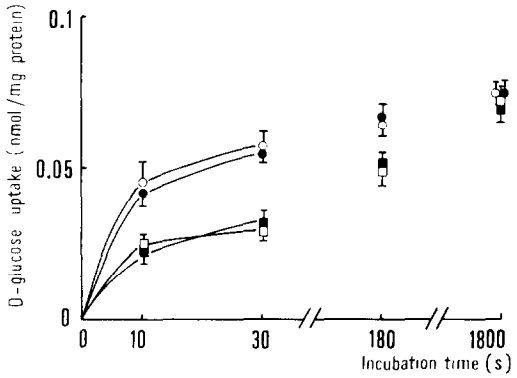


Fig.3. Time course of D-glucose uptake into BBMV at 25°C in the absence of a Na^+ gradient. With NaCl: ○, controls; ●, 1 mM chlorpropham. With KCl: □, controls; ■, 1 mM chlorpropham. Data represent the means ± SE of 4 different preparations. 25 μl of BBMV were preincubated with 25 μl of 200 mM NaCl (or KCl) contained in mannitol Hepes-Tris buffer for 60 min. Chlorpropham was added 10 min before the end of preincubation. Uptake was initiated by adding 50 μl of 0.2 mM D-[^{3}H]glucose and 100 mM NaCl (or KCl) contained in mannitol Hepes-Tris buffer, with or without chlorpropham. Incubation was stopped as described in fig.1.

of spin-labelled BBMV with chlorpropham resulted in an increase of $2T'_{\parallel}$ which means an increase in lipid bilayer order. Fig.4 shows a linear variation of $2T'_{\parallel}$ with chlorpropham concentration. The same concentrations were used to study the effects of the 3 other compounds. The carbamate concentrations producing an increase of $2T'_{\parallel}$ of 1 G were compared. These concentrations (C') were 10 mM for 1, 7.6 mM for 2, 4.5 mM for 3 and 4 mM for 4. The increase of $2T'_{\parallel}$ with N-phenylcarbamates was confirmed again with liposomes prepared from BBMV lipid extract. The mean ± SE (n = 4) of parameter $2T'_{\parallel}$ was for example 40.2 ± 0.1 G for controls and 41.8 ± 0.12 G for 5 mM chlorpropham (not shown). The $2T'_{\parallel}$ values of the BBMV controls and liposome controls are a bit lower but comparable to those given by Hauser et al. [18].

Fig.5 shows the relationship between equi-active (C) concentrations of the various carbamates on D-glucose uptake in the presence of an Na^+ gradient and equi-active (C') concentrations on the value of $2T'_{\parallel}$: $y = 0.126x - 0.325$ (r = 0.987). It should be noted that the C' concentrations are at

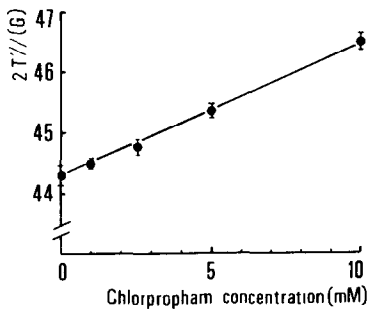


Fig.4. Effect of chlorpropham on the value of $2T'_{\parallel}$ parameter. Data represent means ± SE for 4 different vesicle preparations.

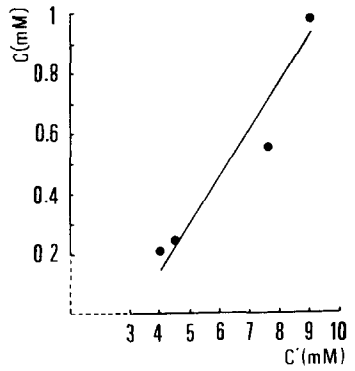


Fig.5. Relation between inhibition of D-glucose uptake and ordering effect of *N*-phenylcarbamates. *C*, concentrations inhibiting 50% of D-glucose uptake; *C'*, concentrations producing an increase of $2T'_{\parallel}$ of 1 G.

least 10-times greater than those active on D-glucose uptake (*C*).

4. DISCUSSION

The results show that the *N*-phenylcarbamates inhibit concentrative Na^+-coupled D-glucose uptake in rat brush border enterocyte vesicles. The lack of modification of the equilibrium level allows the possibility of lysis or alteration of the intravesicular volume, under the action of the carbamates, to be excluded. Furthermore, the kinetics of D-mannose uptake show that the carbamates do not modify passive diffusion.

The active concentrations of the carbamates on concentrative Na^+-coupled D-glucose uptake depend on their partition coefficients and are comparable to those of *n*-aliphatic alcohols of similar partition coefficient causing the same inhibition [7]. The lipophilic character of carbamates seems to be an important factor in determining the intensity of the inhibition but this does not allow elucidation of the exact mechanism by which they act. Measurements of D-glucose uptake under conditions of Na^+ equilibrium show that *N*-phenylcarbamates do not act directly on the carrier but indirectly by favouring the dissipation of the Na^+ electrochemical gradient.

One can wonder about the real interdependence between the activity of D-glucose uptake and the changes in membrane order induced by lipophilic substances. The increase in membrane lipid order by the exogenous substances which we observed has also been shown for other substances [19,20] but the intensity of the ordering or disordering effect seems to depend on the model membrane used [21,22] which shows how complex the problem is. It seems surprising that an increase in lipid order (by *N*-phenylcarbamates) has the same effect on D-glucose uptake as the increase in lipid disorder observed with *n*-aliphatic alcohols [7]. The existence of a state of normal order where D-glucose uptake would be optimal (any increase or decrease in the membrane order causing inhibition) is improbable. Furthermore, although it was demonstrated that a correlation exists between equi-active concentrations of the various carbamates responsible for inhibition and those increasing the membrane order, since the concentrations required for membrane order increase were much greater, the effects on lipid order cannot be responsible for the inhibition of D-glucose uptake. It should also be noted that the measurement of lipid order by ESR depends on the position of the label and only gives an overall evaluation of the physical state of the membrane.

A faster collapse of the Na^+ gradient across the membrane has been suggested to explain the inhibition by ethanol of Na^+-dependent D-glucose uptake [6,23]. This hypothesis was recently confirmed [24] using concentrations which do not modify membrane fluidity. Our results show that, under the action of *N*-phenylcarbamates the faster dissipation of the electrochemical Na^+ gradient alone can explain the inhibition of D-glucose uptake. However, the hydrophobic effect of the carbamates and the increase in membrane lipid order which they cause have no repercussions on the activity of the Na^+-dependent D-glucose carrier.

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