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Interaction of hydrophobic bis (D-mannose) derivatives with adipocyte and erythrocyte sugar transport systems

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The inhibition of sugar uptake by a series of hydrophobic bis(D-mannose) derivatives has been measured in rat adipocytes. When the D-mannose moieties of the bis compounds are separated by a hexane bridge the transport inhibition constant (K_i) is greater than for a decane-bridged molecule. This is probably due to the increased hydrophobicity of the bridge of the decane-bridged compound. The enhancement in affinity due to the second sugar in the bis(D-mannose) derivatives is probably only 2-fold, since half reduction of the bis(D-mannosyloxy)hexane increases K_i approx. 2–3-fold. *N'*-DNP-1,3-bis(D-mannos-4'-yloxy)propyl-2-amine has very high affinity in insulin-treated cells. The affinity is approx. 1000-fold higher than for D-mannose. This enhancement is probably due to the hydrophobicity of the DNP group. The distance from the sugar to the hydrophobic group is important because an increase in K_i occurs if an aminocaproyl spacer is introduced between the DNP group and 1,3-bis(D-mannos-4'-yloxy)propyl-2-amine. Aminocaproyl and glycyl spacers also increase the K_i for NAP derivatives of 1,3-bis(D-mannos-4'-yloxy)propyl-2-amine. Each of the hydrophobic bis(D-mannose) derivatives has a lower K_i in insulin-treated cells. This may be due to an insulin responsive hydrophobic interaction between the hydrophobic portion of the sugar and a hydrophobic domain in the transport system. The inhibition constants for the hydrophobic bis(D-mannose) compounds have also been measured in human erythrocytes.

Introduction

We have recently found that bis(D-mannose) compounds which are crosslinked with a substituted 2-propylamine bridge are very good inhibitors of sugar transport. They are effective outside inhibitors, and because they contain two sugar moieties they do not penetrate the cell membrane either through the facilitated transport system or by diffusion through the membrane lipid [1]. These sugars were synthesised in order to overcome some deficiencies of 4,6-*O*-ethylidene-D-glucose as an outside specific inhibitor. Although ethylideneglucose is a selective inhibitor of the outside site we have shown that it is transported through adipocyte membrane lipid quite rapidly at 37°C [2]. Oka and Czech [3] have confirmed this observation and have used ethylideneglucose very effectively to selectively inhibit photoaffinity labelling by cytochalasin B and to add information on the effect of insulin on the intracellular store of transporters residing in the light microsomal membranes. Photoaffinity labelling of light microsomal membranes was not inhibited by ethylideneglucose

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Abbreviations: FDNP, 5-fluoro-2,4-dinitrophenyl; BMPA, 1,3-bis(D-mannos-4'-yloxy)propyl-2-amine; DNP, 2,4-dinitrophenyl; NAP, 2-nitro-4-azidophenyl; Hepes, 4-(2-hydroxyethyl)1-piperazineethane sulphonic acid.

while this compound was outside the cell but labelling of microsomal membranes was blocked when ethylideneglucose equilibrated through the lipid regions of the plasma membrane. Thus it seemed to us that the improvement in affinity and the reduction in lipid permeability that the bis(D-hexoses) offered was worthy of further investigation so that these compounds would provide a useful addition to the range of outside specific reagents that are available for studying the hexose transport system and for studying the effect of insulin on this system.

In our earlier study [1] we found that FDNP-BMPA had a half saturation value for inhibition of 3-*O*-methyl-D-glucose transport in insulin-treated cells that was approx. 1000-times lower than for the parent sugar D-mannose. We also found that the inhibition constant was higher in the absence of insulin. In an attempt to further understand which portions of the sugar derivatives were responsible for the high affinity and for the insulin dependence of the K_i value some additional derivatives were prepared for the present study. The bridge length and hydrophobicity were varied and inhibition constants for the new derivatives were measured. A hexane-bridged bis(D-mannose) and its partially reduced product (hexane-bridged mannose-mannitol) were prepared in order to evaluate how much of the affinity enhancement of the hexane-bridged bis(D-mannose) was due to the second sugar moiety.

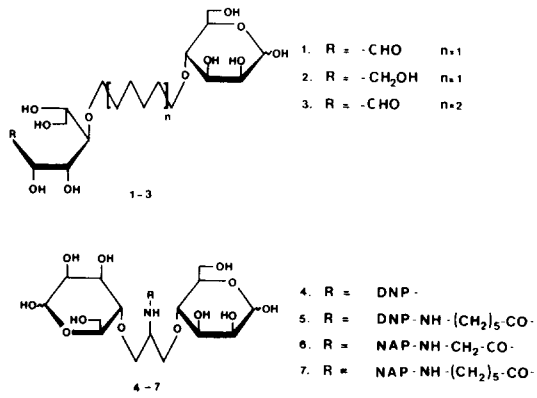
Materials and Methods

Crude collagenase (type 1) was from Worthington Enzymes. Bovine serum albumin was from Sigma. Porcine monocomponent insulin was from Novo Laboratories. Silicone oil was from Hopkins and Williams. Phloretin was from K and K Laboratories. Cytochalasin B was from Aldrich. 3-*O*-Methyl-D-[^{14}C]glucose and D-[1-^{14}C]galactose were from Amersham International.

Synthesis of bis(D-mannose) derivatives

Bis(D-mannos-4'-yloxy)decane was synthesised as previously described [4] for synthesis of 1,6-bis(D-mannos-4'-yloxy)hexane. 1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose was cross-linked with dibromodecane (Aldrich) in dioxan and powdered sodium hydroxide to give 1,10-bis(1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranos-4'-yloxy)decane which was crystallised from ethanol. m.p. = 94–96°C. ^{1}H-NMR data ($CDCl_3$): δ 5.35 (d, 2 H, $J_{1,2}$ 3.0 Hz H-1), 4.60 (dd, 2 H, $J_{5,6}$ 1.5, $J_{5,6'}$ 6.2 Hz, H-5), 4.22 (d, 2 H, H-3), 4.08 (dd, 2 H, $J_{2,3}$ 6.8 Hz, H-2), 3.96 (dd, 2 H, $J_{6,6'}$ 7.5 Hz, H-6), 3.76 (dd, 2 H, H-6'), 3.60 (s, 2 H, H-4), 3.68-3.52 (m, 4 H, $C\underline{H}_2(CH_2)_8C\underline{H}_2$), 1.55 and 1.35 (2s, each 6 H, 2 CMe), 1.90–1.10 (m, 16 H, $CH_2(C\underline{H}_2)_8CH_2$). Mass spectra: electron impact m/z 527 ($M^+ - Me$); chemical ionisation m/z 543 ($M^+ + 1$). Anal. Calcd. for $C_{28}H_{46}O_{10}$: C, 62.01; H, 8.48. Found C, 61.92; H, 8.41.

1,10-Bis(1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranos-4'-yloxy)decane was hydrolysed at approx. 100°C for 3.5 h in 1 M HCl. The solution was neutralised with Amberlite resin IRA-93 (HO^-) and the product 1,10-bis(D-mannos-4'-yloxy)decane was purified by preparative paper chromatography (butanol/ethanol/water (40 : 11 : 19, v/v). Mass spectrum (negative ion f.a.b.) m/z 497 ($M - 1$). The product did not crystallise and the dry oil was used in the transport experiments.



Scheme I. Structures of hydrophobic bis(D-mannose) derivatives. 1, 1,6-bis(D-mannos-4'-yloxy)hexane; 2, 1-(D-mannos-4'-yloxy)-6-(D-mannitol-4-'-yloxy)hexane; 3, 1,10-bis(D-mannos-4'-yloxy)decane; 4, *N'*-DNP-BMPA; 5, *N'*-DNP-aminocaproyl-BMPA; 6, *N'*-NAP-glycyl-BMPA; 7, *N'*-NAP-aminocaproyl-BMPA.

1,6-Bis(D-mannos-4-yloxy)hexane was synthesised as previously described [4]. The crystalline material was reduced to the aldose-alditol and the bis(alditol) in the conventional manner [5]. 2 moles

of $NaBH_4$ per mole of 1,6-bis(D-mannos-4′-yloxy)hexane were added to the sugar solution in ice-cold water. The solution was left to warm to room temperature for 2 h, and was then neutralised with Amberlite MB1. Paper chromatography at this stage showed two products running behind traces of residual 1,6-bis(D-mannos-4′-yloxy)hexane. The slowest product did not stain immediately with alkaline silver nitrate which is consistent with it being the fully reduced alditol. The aldose-alditol gave the characteristic reducing sugar stain. The products were eluted from the chromatogram and after unsuccessful attempts at crystallisation were thoroughly dried to oils.

The other four bis(D-mannose) derivatives used were all obtained from 1,3-bis(D-mannos-4′-yloxy)propyl-2-amine (BMPA). *N*′-NAP-aminocaproyl-BMPA, *N*′-NAP-glycyl-BMPA and *N*′-DNP-aminocaproyl-BMPA were all synthesised from their succinimate esters, while *N*′-DNP-BMPA was synthesised using fluorodinitrobenzene. The method is basically the same for all these derivatives and is described in detail only for *N*′-NAP-aminocaproyl-BMPA.

50 mg of BMPA were dissolved in 1 ml of dimethylsulphoxide and 40 μl of triethylamine. 55 mg of *N*-succinimidyl-6(4′-azido-2′-nitrophenylamino)hexanoate (Pierce) were added and the reaction was left overnight at 50°C. Thin-layer chromatography showed only a single product which contained the chromophore and gave a positive sugar stain. The solution was concentrated to dryness and the product was purified twice by preparative thin-layer chromatography in chloroform/methanol/water (62:25:4, v/v). Hygroscopic crystals of *N*′-NAP-aminocaproyl-BMPA were obtained from 2-propanol 18.6 mg. m.p., approx. 120°C (dec.). Mass spectrum (negative ion f.a.b.) *m/z* 690 (*M*). $\lambda_1 = 260$ nm, $\epsilon_1 = 1.1 \cdot 10^4$; $\lambda_2 = 462$ nm, $\epsilon_2 = 3.4 \cdot 10^3$. *N*′-NAP-glycyl-BMPA gave m.p. approx. 120°C (dec.). Mass spectrum (negative ion f.a.b.) *m/z* 633, 634 (*M* − 1, *M*). $\lambda_1 = 260$ nm, $\epsilon_1 = 1.4 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$; $\lambda_2 = 455$ nm, $\epsilon_2 = 3.0 \cdot 10^3$ M^{-1} cm^{-1}. *N*′-DNP-BMPA gave m.p. = 108–112°C. Mass spectrum (negative ion f.a.b.) *m/z* 581 (*M*). $\lambda_1 = 260$ nm, $\epsilon_1 = 7.8 \cdot 10^3$ M^{-1} cm^{-1}; $\lambda_2 = 363$ nm, $\epsilon_2 = 1.0 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$. *N*′-DNP-aminocaproyl-BMPA gave m.p. = 125–130°C. Mass spectrum (negative ion f.a.b.) *m/z* 694 (*M*). $\lambda_1 = 260$ nm, $\epsilon_1 = 6.8 \cdot 10^3$ $M^{-1} \cdot c,^{-1}$; $\lambda_2 = 362$ nm, $\epsilon_2 = 9.5 \cdot 10^3$ $M^{-1} \cdot cm^{-1}$.

Cell preparations and transport experiments

Human erythrocytes (from blood which had been stored from 1–3 weeks) were prepared as previously described [1]. Transport experiments in erythrocytes (10% cytocrit) were carried out at 20°C in phosphate saline buffer (154 mM NaCl/12.5 mM Na_2HPO_4, pH = 7.4) using 100 μM D-[1-^{14}C]galactose as substrate as previously described [1]. By varying the external inhibitor concentration estimates of the half saturation or K_i values for the bis(D-mannose) derivatives were made. Following these incubations transport was terminated by the addition of ice-cold stopping solution containing 10 μM $HgCl_2$ and 0.3 mM phloretin in phosphate saline buffer. After spinning in a refrigerated bench centrifuge the cell pellets were rewashed in stopping solution and then radiolabelled sugar was extracted into 10% trichloroacetic acid for liquid scintillation counting.

Rat adipocytes were prepared following the method of Rodbell [6] and Gliemann [7] as previously described [1,2]. After digestion of fat tissue in collagenase the separated adipocytes were suspended at 30% cytocrit in Hepes 1% albumin buffer (140 mM Na^+; 4.7 mM K^+; 2.5 mM Ca^{2+}; 1.25 mM Mg^{2+}; 142 mM Cl^-; 2.5 mM $H_2PO_4^-/HPO_4^{2-}$; 1.25 mM SO_4^{2-}; 10 mM Hepes; 1% albumin; pH = 7.4 at 37°C). Transport experiments in adipocytes were carried out at 37°C using 50 μM 3-*O*-methyl-D[^{14}C]glucose as the substrate [8,9]. By varying the inhibitor concentration estimates of the K_i values for the bis(D-mannose) derivatives were made in basal cells and in cells that had been treated with 10 nM insulin. Following these incubations transport was terminated by addition of stopping solution (albumin-free Hepes buffer containing 0.3 mM phloretin). Cells were isolated by spinning through a layer of light silicone oil. Estimates of 'zero uptake' were obtained by measuring the 3-*O*-methyl-D-glucose that was associated with cells that had been treated with 50 μM cytochalasin B.

In both the erythrocyte and the adipocyte experiments the uptake rate constants (v) were calculated from the usual expression $v = t^{-1} \cdot \ln(1$

$-f$), where f is $(cpm_t - cpm_0)/(cpm_\infty - cpm_0)$ [10]. The cpm_0 values were usually 1–2% of cpm_∞, while cpm_t was up to about 50% of cpm_∞ in uninhibited cells. The average rate constant for uninhibited 100 μM D-galactose uptake into erythrocytes at 20°C was 0.08 s^{-1}. The average rate constants for 50 μM 3-*O*-methyl-D-glucose uptake into adipocytes at 37°C were 0.22 s^{-1} in insulin-treated cells and 0.0037 s^{-1} in basal cells. Since the substrate concentration is low compared with the substrate K_m value then K_i for the inhibitor is calculated from $K_i = I/(v_0/v - 1)$, where I is the inhibitor concentration and v_0 and v are the uninhibited and inhibited rate constants, respectively [11]. Results are expressed throughout as mean ± S.E.

Results and Discussion

The introduction of a hexane bridge between two D-mannose moieties gives 1,6-bis(D-mannos-4-yloxy)hexane. This compound has a half saturation constant (K_i) of 1.23 ± 0.07 mM for inhibition of 3-*O*-methyl-D-glucose uptake in insulin-treated rat adipocytes (Fig. 1). This is a considerable improvement in affinity compared with D-mannose which has a $K_i = 22.12 \pm 2.27$ mM (Table I). When 1,6-bis(D-mannos-4'-yloxy)hexane is half reduced with $NaBH_4$ the resulting derivative has D-mannose at one end and D-mannitol at the other end of the molecule. This compound 1-(D-mannos-4'-yloxy)-6-(D-mannitol-4'-yloxy)hexane has a $K_i = 3.51 \pm 0.26$ mM, which is approx. 2–3-times that of the parent bis(D-mannosyloxy)hexane compound. It seems likely that this change in affinity is a statistical factor arising from the half loss of D-mannose in the molecule. It follows that the remaining enhancement in affinity of 1,6-bis(D-mannos-4'-yloxy)hexane compared with D-mannose is likely to be due to the hydrophobicity of the bridge. We have previously shown that certain 1,3-bis(D-mannos-4'-yloxy)propyl-2-amine derivatives show an insulin-dependent half-saturation constant. This is also shown for 1,6-bis(D-mannos-4'-yloxy)hexane (Fig. 1). In the absence of insulin the K_i value rises to 3.22 ± 0.26 mM which is 2.6-fold higher than in insulin-treated cells. In an attempt to determine which part of the bis(D-mannose) derivatives was responsible for this effect we have compared the half-saturation constant for D-mannosyloxy-D-mannitolyloxyhexane in the presence and in the absence of insulin. The half-saturation constant is still insulin-dependent although the magnitude of the change due to insulin is somewhat less at 1.8–2.0-fold (two separate experiments). Thus it seems that the hydrophobicity of the bridge is mainly responsible for the high affinity for the bis(D-mannose) derivatives and also for the insulin dependence of the half-saturation constant. The reason why we have not previously detected this insulin-dependent effect may be because the hydrophobic substitution has to be in a specific position on the sugar. Also, the magnitude of the insulin dependence does seem to be slightly correlatable with the effectiveness of the hydrophobic substitution. Thus our most successful high-affinity compounds to date are *N'*-DNP-BMPA (see below) and *N'*-FDNP-BMPA [1] which both have K_i values of approx. 22 μM in insulin-treated cells. In the absence of insulin the K_i values rise to 80–100 μM which is an approx. 4-fold change. This is a significantly larger change than that which occurs in the half-saturation value for D-mannosyloxy-D-mannitol-yloxyhexane, where the magnitude of the insulin effect on the half saturation value was only

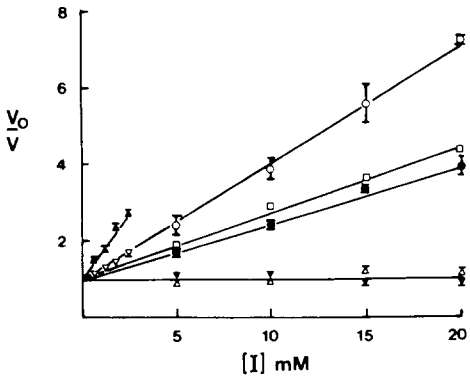


Fig. 1. The inhibition of the uptake of 50 μM 3-*O*-methyl-D-glucose in adipocytes at 37°C by 1,6-bis(D-mannos-4'-yloxy)hexane in insulin-treated cells (▲) and in basal cells (▽) and by 1-(D-mannos-4'-yloxy)-6-(D-mannitol-4'-yloxy)hexane in insulin-treated cells (○) and in basal cells (●). 1,6-bis(D-mannitol-4'-yloxy)hexane gives no inhibition neither in the presence (▼) nor in the absence (△) of insulin. The inhibition of 100 μM D-galactose uptake in erythrocytes at 20°C by 1,6-bis(D-mannos-4'-yloxy)hexane is also shown (□).

TABLE I

HALF-MAXIMAL INHIBITION CONSTANTS FOR HYDROPHOBIC BIS(D-MANNOSE) DERIVATIVES

Results are mean $K_i \pm$ S.E. (n = eight in all cases).

	K_i (μM)		
	Adipocytes		Erythrocytes
	+10 nM insulin	Basal cells	
1,6-Bis(D-Mannos-4'-yloxy)hexane	1230 ± 70	3220 ± 260	5500 ± 140
1-(D-Mannos-4'-yloxy)-6-(D-mannitol-4'-yloxy)hexane	3510 ± 260	7040 ± 440	-
1,10-Bis(D-mannos-4'-yloxy)decane	126 ± 8	315 ± 28	849 ± 44
N'-DNP-BMPA	22 ± 1.6	80 ± 5.7	294 ± 11
N'-DNP-aminocaproyl-BMPA	265 ± 38	772 ± 43	1520 ± 50
N'-NAP-glycyl-BMPA	319 ± 32	898 ± 79	589 ± 8
N'-NAP-aminocaproyl-BMPA	261 ± 10	652 ± 21	587 ± 25
D-Mannose	22120 ± 2270	18690 ± 1040	-

1.8–2.0-fold. In order to check that the insulin dependence of the K_i value was not due to a difference in experimental design in the insulin versus noninsulin experiments, we have measured the K_i for *N'*-DNP-BMPA after a 3 min preincubation with insulin-treated cells. This gives a time of incubation with the inhibitor which is comparable with the longer (3 min) time-courses used for estimating transport rates in basal cells. The K_i was 23.75 ± 1.61 μM, which is not significantly different from the K_i value of 22 ± 1.6 μM (Table I) estimated over the normally short (3 s) time-course used in insulin-treated cells.

When 1,6-bis(D-mannosyl-4'-yloxy)hexane is completely reduced the resulting compound has negligible affinity for the transport system neither in the presence nor in the absence of insulin (Fig. 1). This result indicates that the hydrophobic bridge between two hydrophilic polyols is not sufficient to produce transport inhibition and at least one half of the molecule has to contain a sugar moiety which is capable of binding to the transport system.

The importance of the hydrophobicity of the bridge to transport inhibition when D-mannose is present in the molecule is indicated by a comparison of the affinity for 1,6-bis(D-mannos-4'-yloxy)hexane and for 1,10-bis(D-mannos-4'-yloxy)decane. The latter compound has a $K_i = 126 \pm 8$ μM in insulin-treated cells (Fig. 2), which is approx. 10-fold lower than the K_i value for the compound with the shorter bridge. This change in inhibition constant would correspond to an approx. 1.8-fold increase in inhibition constant for each additional -CH_2-group. In the absence of insulin the inhibition constant is 315 ± 28 μM for this derivative. Although one might imagine that the introduction of a 10-carbon alkyl chain would give a detergent-like cell disruptive compound we find no evidence of cell lysis due to this sugar. We find that the cellular volume occupied by the radiolabelled 3-*O*-methyl-D-glucose is unaffected by the presence of all the tested concentrations of

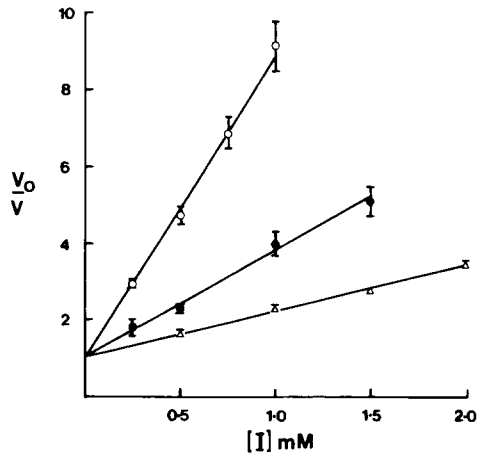


Fig. 2. The inhibition of 50 μM 3-*O*-methyl-D-glucose uptake in adipocytes at 37°C by 1,10-bis(D-mannos-4'-yloxy)decane in insulin-treated cells (○) and in basal cells (●). The inhibition of 100 μM D-galactose uptake in erythrocytes at 20°C by this compound is also shown (△).

1,10-bis(D-mannos-4′-yloxy)decane. Also the behaviour of this sugar in chromatographic systems is inconsistent with the molecule as a whole being very hydrophobic. The hydrophobicity of the bridge is presumably balanced by the hydrophilicity of the second sugar moiety.

The alkyl chain bridged D-mannose results indicate that a hydrophobic bridge substituent is an important requirement for good inhibition. However, the hydrophobic substituent may have to be in a specific position relative to the sugar moiety which is located in the transport binding site. The reason for this suggestion is based on a comparison of the K_i value for the 1,3-bis(D-mannos-4′-yloxy)propyl-2-amine (BMPA) derivatives *N*′-DNP-BMPA and *N*′-DNP-aminocaproyl-BMPA. *N*′-DNP-BMPA has an affinity constant ($K_i = 22.0 \pm 1.6$ μM in insulin-treated cells) which is very similar to that previously measured for FDNP-BMPA ($K_i = 21$ μM [1]). *N*′-DNP-aminocaproyl-BMPA has a 6-carbon aminohexanoate spacer between the DNP moiety and the amino group of the bis(D-mannosyloxy)propylamine bridge. We had originally assumed that introducing the additional hydrophobic spacer into the molecule might well reduce the half-saturation constant because of an enhanced hydrophobic interaction with the transport system. However, the introduction of the additional hydrophobic spacer has an effect which is the opposite to the predicted result. The K_i rises to 265 ± 38 μM for *N*′-DNP-aminocaproyl-BMPA. This large increase in inhibition constant may mean that the DNP group has to be close to the sugar to be most effective. This result would thus be consistent with there being a hydrophobic domain of specific peptide side groups in the transport system which binds the DNP group and which is close to a more hydrophilic pocket which binds the sugar moiety. The result is less easy to interpret in terms of a nonspecific or delocalised hydrophobic interaction with membrane lipid since the DNP group in *N*′-DNP-aminocaproyl-BMPA might be expected to be more, not less, effective in interacting with such a region. The reason for the proposed hydrophobic peptide domain having a more pronounced affinity enhancing effect in the presence of insulin may be that there is an insulin dependent change in the position of this hydrophobic domain relative to the position of the hydrophilic domain which binds the sugar moiety. It is, however, clear that the magnitude of the insulin effect on K_i is in all cases very small when compared with effect of insulin on the uninhibited transport rate constant. There is an approx. 60-fold change in the rate constant for 3-*O*-methyl-D-glucose transport in these experiments and this effect is presumably

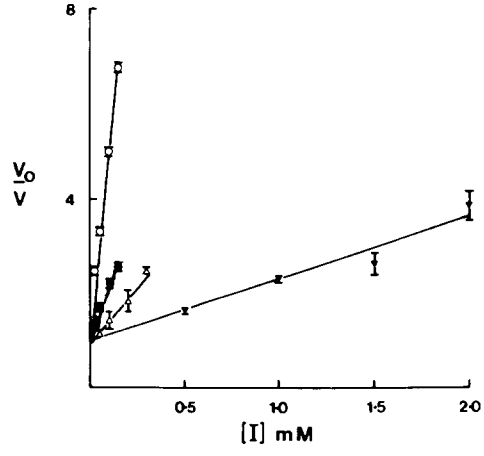


Fig. 3. The inhibition of 50 μM 3-*O*-methyl-D-glucose uptake in adipocytes at 37°C by *N*′-DNP-BMPA in insulin-treated cells (○) and in basal cells (●) and by *N*′-DNP-aminocaproyl-BMPA in insulin-treated cells (△) and in basal cells (▼).

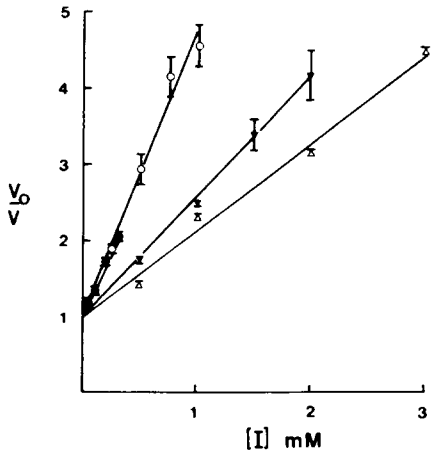


Fig. 4. The inhibition of the uptake of 50 μM 3-*O*-methyl-D-glucose in adipocyes at 37°C by *N*′-NAP-aminocaproyl-BMPA in insulin-treated cells (○) and in basal cells (▼) and by *N*′-NAP-glycyl-BMPA in insulin-treated cells (●) and in basal cells (△).

due to the recruitment of new transport sites to the plasma membrane in the presence of insulin [12–14].

Fig. 4 shows the affinities for BMPA derivatives which have been substituted by nitroazidophenyl (NAP) groups. *N'*-NAP-glycyl-BMPA and *N'*-NAP-aminocaproyl-BMPA have similar K_i values. Both gave K_i values of about 300 μM in insulin-treated cells (Table I). Our previous study [1] showed that *N'*-NAP-BMPA has an apparent half saturation constant of 46 μM in insulin-treated cells. Thus introducing either an aminocaproyl spacer or a shorter glycyl spacer lowers the affinity by about 6–7-fold. Therefore the *N'*-NAP-glycyl and the *N'*-NAP-aminocaproyl derivatives of BMPA are unlikely to be of use in specific photoaffinity labelling of the transporter since high affinity is an important requirement in such experiments with nontransported transport inhibitors [15–17].

The half-saturation constants for the hydrophobic bis(D-mannose) compounds have also been measured in human erythrocytes at 20°C. Here the substrate used was 100 μM D-galactose. The relative affinities are very similar in adipocytes and erythrocytes but the erythrocyte K_i values are roughly 2-fold higher than in adipocytes in the absence of insulin. In erythrocytes increasing the bridge length between the two D-mannose moieties lowers the K_i value from 5.5 ± 0.14 mM for 1,6-bis(D-mannos-4'-yloxy)hexane (Fig. 1) to 849 ± 44 μM for 1,10-bis(D-mannos-4'-yloxy)decane (Fig. 2). Introducing the aminocaproyl spacer between a DNP group and the propyl-2-amine bridge of BMPA increases the inhibition constant from 294 ± 11 μM for *N'*-DNP-BMPA to 1.52 ± 0.05 mM for *N'*-DNP-aminocaproyl-BMPA (Fig. 5). The K_i of *N'*-NAP-BMPA in erythrocytes is 348 μM [1] while *N'*-NAP-aminocaproyl-BMPA and *N'*-NAP-glycyl-BMPA both have K_i values of approx. 590 μM (Fig. 5). Although the K_i values are generally higher in erythrocytes than in adipocytes the relative affinities shown by erythrocytes for most of the hydrophobic bis(D-mannose) derivatives are similar to those of adipocytes. However, there are some slight differences and the NAP compounds have lower K_i values in erythrocytes than in noninsulin-treated adipocytes. We have previously shown that the azidosalicylamide derivative of BMPA also has a lower K_i in erythrocytes than in adipocytes. These minor differences in affinity are consistent with the postulate that there are some minor differences in the spatial requirements of the erythrocyte compared with the adipocyte systems [19].

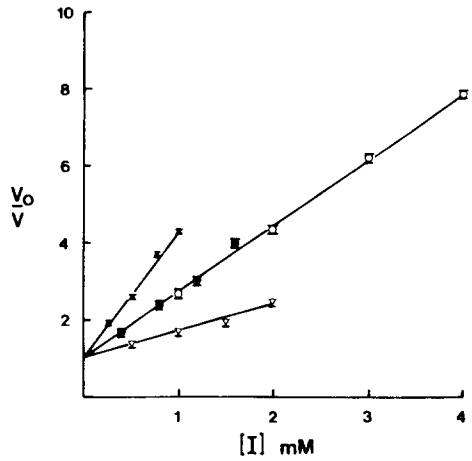


Fig. 5. The inhibition of the uptake of 100 μM D-galactose in erythrocytes at 20°C by *N'*-DNP-BMPA (△), by *N'*-NAP-aminocaproyl-BMPA (●), by *N'*-NAP-glycyl-BMPA (○) and by *N'*-DNP-aminocaproyl-BMPA (▽).

In conclusion it seems that the results of this study on the inhibition constants for hydrophobic bis(hexoses) in adipocytes and erythrocytes confirm that the high affinity which is shown by these compounds make the bis(hexoses) a useful addition to the range of reagents that can be used in studying sugar transport.

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