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Effect of chemotactic factors on hexose transport in polymorphonuclear leucocytes

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Transport of the nonmetabolizable glucose analogue, 3-*O*-methylglucose, was assessed in human polymorphonuclear leucocytes with or without the chemotactic peptide *N*-formylmethionylleucylphenylalanine (fMet-Leu-Phe). The peptide increased entry of labelled 3-*O*-methylglucose about 5-fold and the intracellular distribution space about 70%. The half-time of equilibration was 3 s in the treated cells. Similar effects were observed with zymosan-treated serum (containing the chemotactic factor C5a), with arachidonic acid, calcium ionophore A23187 and phorbol myristate acetate. However, the chemotactic protein, thrombin, had no effect, even though binding to high-affinity receptors was demonstrated. K_m for zero-trans entry of 3-*O*-methylglucose was about 1 mM and fMet-Leu-Phe increased V_{max} from 5 to about 25 amol · s^{-1} · $cell^{-1}$. Similar values were obtained from incubations for a few seconds with glucose and 2-deoxyglucose. The rate of 2-deoxyglucose uptake (8 min incubations) was limited by the transport step at substrate concentrations lower than approx. 0.1 mM, whereas the phosphorylation step became rate-limiting at higher concentrations. Thus, 2-deoxyglucose uptake can only be taken as a measure of transport at a tracer concentration. It is concluded that chemotactic factors can, but do not necessarily, increase the maximal transport velocity of hexoses entering the polymorphonuclear leucocyte via the glucose transporter.

Introduction

Previous studies have shown that chemotactic factors such as complement component C5a [1] and the synthetic oligopeptide, fMet-Leu-Phe [2], enhance uptake of 2-deoxyglucose in human polymorphonuclear leucocytes. A similar effect is obtained with arachidonic acid, which is released upon interaction with fMet-Leu-Phe [2,3] and with stimulators of protein kinase C such as phorbol esters. 2-Deoxyglucose accumulates in the cells as 2-deoxyglucose phosphate and is not metabolised to any major extent beyond the hexokinase step. Uptake of labelled 2-deoxyglucose is inhibited by cytochalasin-B [2], an inhibitor of the glucose transporter, and the uptake is therefore likely to reflect the transport as long as the hexokinase can phosphorylate all the sugar entering the cell. However, the capacity of hexokinase may be exceeded under various conditions and particularly at high sugar concentrations. Therefore, uptake of 2-deoxyglucose, as measured for 15–30 min [1–4] cannot always be taken as a measure of transport.

We have previously shown that exchange of the nonmetabolizable glucose analogue, 3-*O*-methylglucose, between the incubation medium and the

Abbreviations: fMet-Leu-Phe, formylmethionylleucylphenylalanine; PMA, phorbol 12-myristate 13-acetate.

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intracellular water of resting polymorphonuclear leucocytes occurs via facilitated diffusion and with a K_m of about 4 mM [5]. The purpose of the present study was to characterise the hexose transport system in resting cells and in cells stimulated with chemotactic factors.

Materials and Methods

3-*O*-[^{14}C]Methyl-D-glucose ($2.0 \cdot 10^{12}$ Bq/mol), 2-deoxy-D-[U-^{14}C]glucose ($1.2 \cdot 10^{13}$ Bq/mol), D-[U-^{14}C]glucose ($10 \cdot 10^{13}$ Bq/mol) and ^3H_2O ($1.1 \cdot 10^7$ Bq/mol) were purchased from Amersham International. L-[1-^{3}H]Glucose ($7 \cdot 10^{14}$ Bq/mol) was from New England Nuclear. Unlabelled glucose analogues, fMet-Leu-Phe, zymosan, PMA and arachidonic acid were from Sigma, cytochalasin-B was from Aldrich, phloretin from K & K Laboratories and dextran T 110 and Percoll were from Pharmacia. Other reagents were analytical grade. Zymosan-treated serum was prepared by the addition of zymosan (25 mg/ml) to normal human serum for 30 min at 37 °C followed by centrifugation to remove the particulate material. ^{125}I-labelled thrombin (about 0.7 mol iodine/mol thrombin) was kindly provided by Dr. O. Sonne, Institute of Physiology, University of Aarhus.

Polymorphonuclear leucocytes were prepared without exposure to hypotonic medium as described previously [5]. In brief, 450 ml fresh blood was mixed with 30 ml 0.9% NaCl containing 1500 units of heparin followed by centrifugation for 15 min at $6000 \times g$. 20 ml of the top layer was recovered ('buffy coat') and mixed with 5 ml of 10% dextran in 0.9% NaCl with 400 units heparin/ml. After sedimentation of most of the erythrocytes for 30 min at 37 °C, the supernatant was centrifuged for 4 min at $150 \times g$ followed by resuspension of the sedimented cells in buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5 mM $NaHCO_3$ and 31 mM Mops (pH 7.4) at 37 °C. The centrifugation was repeated, the cells were resuspended in 2.5 ml buffer, layered on top of a preformed linear Percoll gradient adjusted to 1.044–1.097 kg/l and centrifuged for 10 min at about $4000 \times g$. The polymorphonuclear leucocytes were recovered from the band of highest density. Erythrocytes stuck to the bottom of the tube, whereas monocytes and lymphocytes were in bands of lower densities. The purity of the leucocytes was 96–99% with 1–4% lymphocytes, and more than 99% of the cells excluded Trypan blue.

Incubations were carried out in 4.5 ml round-bottom mini scintillation vials (Hansac Plastic, Hasselager, Denmark). In transport experiments, incubations were carried out for a few seconds in order to measure initial uptake rates [5]. The incubations were initiated by squirting 45 µl cell suspension, containing about $5 \cdot 10^6$ cells at 37 °C on to 15 µl isotope-containing buffer placed in the bottom of the incubation vial. In some experiments (zero trans entry) unlabelled glucose or glucose analogue was in the 15 µl buffer at four times the final concentration. In other experiments (equilibrium exchange) the cells were suspended and preequilibrated for 15 min in buffer containing 3-*O*-methylglucose at the appropriate concentration. In this case, the 15 µl buffer with isotope contained the glucose analogue at the same concentration [6]. Timing of the incubations were carried out using a metronome and they were stopped by the addition of 3.5 ml 0.3 mM phloretin in buffer containing 0.1 µM $HgCl_2$. The cells were immediately pelleted by centrifugation at $4000 \times g$ for 1 min, the supernatant was discarded and the procedure was repeated once. Finally, 2.5 ml of scintillation fluid was added and the radioactivity associated with the cell pellet was determined. Blank values, as determined by the addition of stopping solution before the cells, were subtracted from all measurements, and they contained 6–10% of the counts present in the cell pellet when cells had equilibrated with labelled 3-*O*-methylglucose for 3 min. In some experiments the cell pellet was resuspended in 1 ml H_2O, boiled for 5 min and centrifuged. The supernatant was applied to a 0.7×4 cm anion-exchange column (Bio-Rad AG 1-X8) and labelled methylglucose was removed by washing with 2.5 ml 1 mM methylglucose in water. Phosphorylated methylglucose initially retained on the column was removed by 12.5 ml 0.2 M formic acid in 0.5 M ammonium acetate [5,7].

Uptake experiments with 2-deoxyglucose were performed with incubations for several minutes in a volume of 150 µl. The incubations were stopped by the transfer of 100 µl aliquots to 500 µl micro-

fuge tubes containing 100 μl dibutylphthalate/dinonylphthalate (3 : 1, $d = 1.0245$) followed by centrifugation at about $10000 \times g$ for 40 s in a Beckman microfuge [6,8]. The tube was cut through the oil layer and the cell pellet was transferred for determination of radioactivity. Extraction of the scintillation fluid for at least 24 h was necessary to obtain stable activity. The same procedure was used to determine intracellular distribution spaces for ^{14}C-labelled methylglucose and $^{3}H_2O$ using ^{3}H-labelled L-glucose as a marker for the extracellularly trapped volume in the cell pellet. Incubations with ^{125}I-labelled thrombin were carried out in a volume of 300 μl and the cell pellet was recovered as described for uptake of 2-deoxyglucose.

The results are representative of at least three experiments unless stated otherwise.

Results

Table I shows the effect of 20 nM fMet-Leu-Phe on the intracellular distribution space for $^{3}H_2O$ and ^{14}C-labelled 3-*O*-methyl-D-glucose after incubation for 2 min. In resting cells this distribution space (i.e., methyl-D-glucose minus L-glucose space in the cell pellet) was about 70% of the intracellular distribution space for $^{3}H_2O$ (i.e., intracellular water space), in agreement with previous results [5]. Treatment with the chemotactic peptide, fMet-Leu-Phe, caused a slight increase in the intracellular water space, indicating an increase in cell volume. The intracellular apparent distribution space for methylglucose increased markedly and to a level not distinguishable from that of $^{3}H_2O$. The increase in the apparent distribution space for methylglucose was in fact due to methylglucose and not to methylglucose phosphate, cf. legend to Table I. Thus, treatment with fMet-Leu-Phe caused both a minor (about 20%) increase in cell volume and a larger (about 70%) increase in the intracellular distribution space for the glucose analogue. This implies that most of the latter increase is caused by distribution of meth-

TABLE I

DISTRIBUTION SPACES FOR $^{3}H_2O$ AND ^{14}C-LABELLED 3-*O*-METHYL-D-GLUCOSE IN POLYMORPHONUCLEAR LEUCOCYTES

The cells ($1 \cdot 10^8$/ml) were preincubated for 10 min at 37 °C. fMet-Leu-Phe (final concentration 20 nM) was dissolved to give a final concentration of 0.2% in dimethyl sulfoxide and the control cells contained this vehicle at the same concentration. Other experiments showed that dimethyl sulfoxide alone did not affect the distribution spaces. The cells were incubated for 2 min with the tracers followed by centrifugation through oil. Anion-exchange chromatography showed that less than 2% of the ^{14}C activity in the cell pellet from both fMet-Phe-treated and untreated cells was retained on the column. The results represent the mean values of six separate incubations ±1 S.D. The distribution space for ^{3}H-labelled L-glucose in the cell pellet (corresponding to about 50 fl/cell) was measured in all experiments and the values were subtracted to give the intracellular distribution spaces. The differences a vs. b, a vs. c and b vs. d were seen in all six experiments.

	Intracellular distribution spaces (fl/cell)	
	$^{3}H_2O$	3-*O*-[^{14}C]methylglucose
No treatment	a 273 ± 11	b 198 ± 13
fMet-Leu-Phe	c 330 ± 17	d 342 ± 28

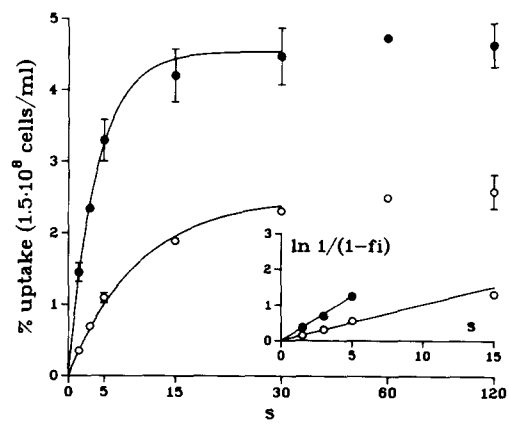

Fig. 1. Time course of 3-*O*-[^{14}C]methylglucose uptake into resting (○——○) and fMet-Leu-Phe-treated (●——●) cells. The concentration was $2 \cdot 10^3$ Bq/tube ($3.4 \cdot 10^7$ Bq/l which equals about 6 μM). The blank value was determined by first adding a small volume of stopping solution to the 15 μl ^{14}C-labelled 3-*O*-methylglucose followed by 45 μl cell suspension and the remaining stopping solution. It was not different from that observed with ^{3}H-labelled L-glucose (i.e., trapped extracellular volume in the cell pellet) and represented about 5% of the uptake at 2 min in the treated cells. The ordinate shows the percentage of the tracer in the incubation present in the intracellular compartment, i.e., after subtraction of the blank value. The points are the means of three replicates ±1 S.D. when this exceeds 5% and the curves are the best-fitting exponentials. The inset shows the rate constants of entry as 0.090 s^{-1} for resting and 0.243 s^{-1} for treated cells.

ylglucose into an intracellular compartment not accessible to the glucose analogue in the resting cell. Similar results were obtained in analogous experiments with 10 μM arachidonic acid, 100 nM of the Ca^{2+} ionophore A23187, and with zymosan-treated serum diluted 1 : 100, whereas dilute normal serum had no effect (data not shown).

Fig. 1 shows the time-course of uptake of labelled methylglucose into resting and fMet-Leu-Phe-treated cells. Both uptake curves are approximately exponential and the initial uptake is about 4-times higher in the treated cells than in the resting cells. The rate constant of entry is also increased, although only by a factor of 2–3, due to the higher distribution volume for methylglucose in the treated cells. Uptake in the presence of 20 μM cytochalasin B was almost entirely blocked in the stimulated cells (data not shown) as previously demonstrated in resting cells [5]. In eight similar experiments 20 nM fMet-Leu-Phe caused a 5.1 ± 1.1. (1 S.D.) fold increase in the initial methylglucose uptake and all experiments showed both an increase in the rate constant of entry and in the apparent distribution volume. Addition experiments showed that 20 nM was enough to elicit the maximal response of fMet-Leu-Phe and that the half-maximal response was obtained with approx. 3 nM peptide. Further experiments (not shown) demonstrated that incubation of the cells with zymosan-treated serum diluted 1 : 100, 100 nM of the Ca^{2+} ionophore A23187, 10 μM arachidonic acid or 2 nM PMA gave results similar to those obtained with the chemotactic peptide. Other experiments showed that insulin had no effect.

The following experiments were designed to explore the concentration dependence of methylglucose transport in resting and fMet-Leu-Phe-stimulated cells. Incubation times were adjusted to give about 20% of the uptake at equilibrium and this was regarded as a measure of initial velocities. Thus, with very low methylglucose concentrations, resting cells were incubated for about 4 s and treated cells for about 1.5 s (cf. Fig. 1). Fig. 2A shows the concentration dependence of 3-*O*-methylglucose exchange (i.e., same concentration of the sugar analogue outside and inside, tracer on the outside). K_m for the resting cells was 3–4 mM, in agreement with previously published data [5], and stimulation caused a decrease in K_m to around 1 mM. Fig. 2B shows the equivalent data for zero trans experiments (i.e., sugar analogue initially only on the outside) and both resting and stimu-

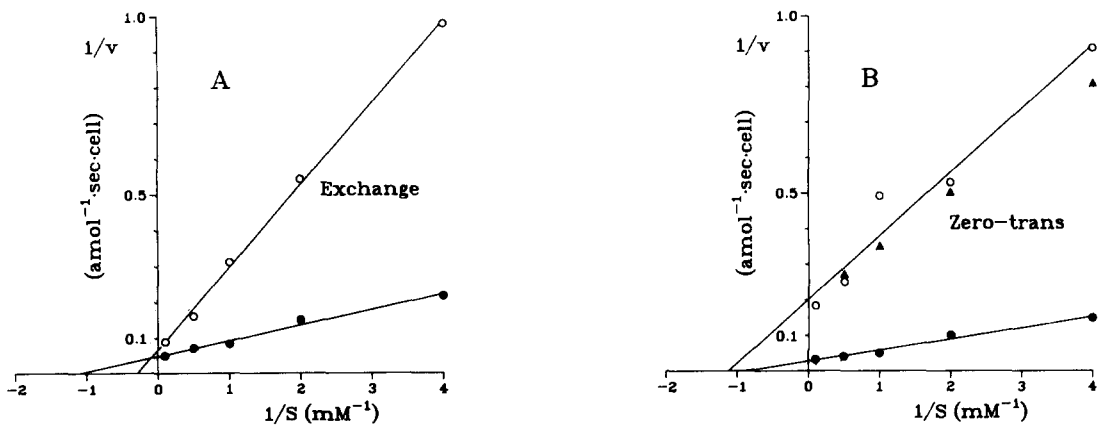

Fig. 2. Concentration dependence of 3-*O*-methylglucose transport. (A) Exchange. The cells were preincubated for 15 min with unlabelled 3-*O*-methylglucose as indicated in reciprocal on the abscissa. The incubations were initiated by the addition of $2 \cdot 10^3$ Bq ^{14}C-labelled 3-*O*-methylglucose and v was calculated from the initial velocity of entry (cf. Fig. 1). The regression lines were calculated by least-square analysis. The values for resting cells (○———○) were: K_m (negative reciprocal x-intercept), 3.48 mM; V_{max} (reciprocal y-intercept), 15.2 amol·s^{-1}·cell^{-1}. The values for stimulated cells (●———●) were $K_m = 0.91$ mM; $V_{max} = 20.8$ amol·s^{-1}·cell^{-1}. (B) Zero trans. The incubations were initiated by the addition of both labelled and unlabelled 3-*O*-methylglucose to the outside medium. Resting cells (○———○) $K_m = 0.90$ mM; $V_{max} = 5.0$ amol·s^{-1}·cell^{-1}. Stimulated cells (●———●) $K_m = 1.1$ mM; $V_{max} = 33.0$ amol·s^{-1}·cell^{-1}. The triangles show transport in the presence of 10 nM thrombin.

TABLE II

KINETIC PARAMETERS FOR TRANSPORT OF 3-*O*-METHYL-D-GLUCOSE

Summary of experiments as those shown in Fig. 2A and B. Mean values ±1 S.D. with the number of experiments indicated in brackets.

	Exchange (7)		Zero-trans (9)	
	K_m (mM)	V_{max} (amol·s^{-1}·cell^{-1})	K_m (mM)	V_{max} (amol·s^{-1}·cell^{-1})
No treatment	3.9±0.4	21.8±3.8	0.9±0.2	4.8±0.9
20 nM fMet-Leu-Phe	1.0±0.3	30.2±5.1	1.1±0.2	26.1±4.0

lated cells showed K_m values of around 1 mM. Table II summarises the results of several experiments. The following can be concluded: fMet-Leu-Phe causes a decrease in K_m for 3-*O*-methylglucose exchange; a minor increase in V_{max} cannot be excluded. There is an increase in V_{max} for zero-trans uptake which appears to account for the stimulatory effect of fMet-Leu-Phe.

Some experiments were carried out using zymosan-treated serum and the effect on K_m and/or V_{max} was essentially the same. It seems possible, therefore, that stimulation of hexose transport might be a general property of factors with chemotactic effect on polymorphonuclear leucocytes. We decided to use thrombin, since it was recently reported that this proteinase has a chemotactic effect on human polymorphonuclear leucocytes indistinguishable from that of fMet-Leu-Phe [9]. However, 10 nM thrombin had no effect on 3-*O*-methylglucose transport, as seen from Fig. 2B or on the distribution space of this glucose analogue. Whereas receptors for fMet-Leu-Phe in the leucocytes are well-characterised [10,11], receptors for thrombin have not been demonstrated. We therefore wanted to ascertain that thrombin actually was bound. Fig. 3, inset, shows that binding of ^{125}I-labelled thrombin occurs rapidly at 4°C with plateau by 15–30 min. Binding was essentially complete by 2 min at 37°C (data not shown). It appears from the concentration dependence (Fig. 3) that 10 nM unlabelled thrombin provides a high occupancy of the receptors and probably a complete occupancy of the high affinity receptors. Nevertheless, thrombin did not increase 3-*O*-methylglucose transport, cf. Fig. 2B. Thus, receptor occupancy with chemotactic peptides or proteins can, but does not necessarily, cause stimulation of the glucose transport.

We next attempted to measure the transport rates of the metabolizable sugars, D-glucose and 2-deoxy-D-glucose. It is assumed that initial uptake (zero-trans conditions) is a good measure of the transport rate as long as the uptake corresponds to only 20–25% of the intracellular distribution space for 3-*O*-methylglucose. The only way a large error can occur is if the sugar is converted to metabolites which are very rapidly released

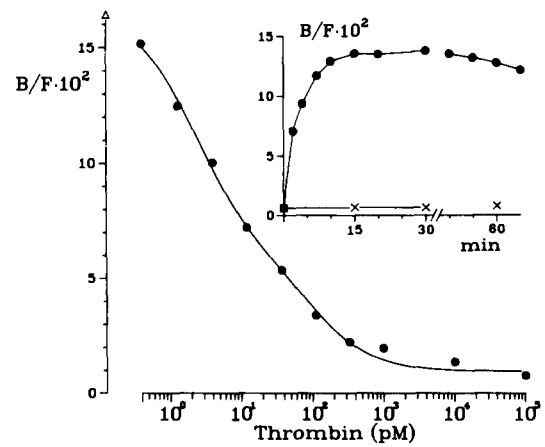

Fig. 3. Specific binding of thrombin to polymorphonuclear leucocytes ($1 \cdot 10^7$/ml). The inset shows the time-course at 4°C of ^{125}I-labelled thrombin (50 Bq/ml) association with the cell pellet either in the absence (●———●) or presence (×———×) of 1 μM unlabelled thrombin. The main graph shows the effect of unlabelled thrombin (●———●) on the binding of tracer alone (△) with the data corrected for counts in the cell pellet in the presence of 1 μM thrombin. Scatchard analyses showed that most of the tracer binding was accounted for by receptors with a dissociation constant of about 9 pM and that there was an additional minor binding due to sites with very low affinity.

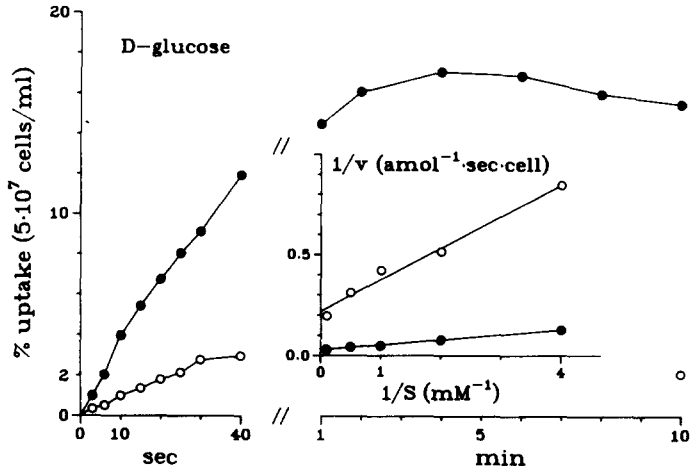

Fig. 4. Transport and uptake of glucose. The inset shows data analogous to those presented in Fig. 2B with D-glucose as tracer and unlabelled substrate. The calculated values are: for untreated cells (○———○) $K_m = 1.09$ mM, $V_{max} = 4.6$ amol·s^{-1}·cell^{-1}; and for fMet-Leu-Phe-treated cells (●———●) $K_m = 0.93$ mM, $V_{max} = 37.0$ amol·s^{-1}·cell^{-1}. The main graph shows the uptake of 5 μM labelled glucose in untreated (○———○) and fMet-Leu-Phe-treated cells (●———●).

from the cells. Thus, if labelled D-glucose was converted to CO_2 immediately after its entry, then the label would be lost from the cell pellet. However, we found only negligible $^{14}CO_2$ production in 1 min incubations (data not shown). The inset of Fig. 4 shows the result of an experiment analogous to that shown in Fig. 2B, but with D-glucose. The result of several experiments gave values indistinguishable from those obtained with 3-*O*-methylglucose. Fig. 4 also shows the expanded time-course for glucose uptake at tracer concentration. Uptake is approximately linear for 40 s and transport may therefore be rate-determining for the uptake at very low substrate concentrations. Uptake of labelled glucose in fMet-Leu-Phe-stimulated cells (40 s incubations) was inhibited half-maximally by about 0.2 mM glucose (data not shown), a concentration much below K_m for the glucose transport. Thus, glucose transport probably ceases to be rate-determining for the uptake at glucose concentrations of 0.2 mM or higher.

These points are perhaps analysed more clearly using 2-deoxyglucose, which is not metabolised beyond the hexokinase step. We first performed experiments analogous to those shown in Fig. 2B and Fig. 4, inset. These results, shown in Table III, are indistinguishable from those with glucose and 3-*O*-methylglucose. Fig. 5 shows that uptake of ^{14}C-labelled 2-deoxyglucose was linear for 8 min. Other experiments with more concentrated cells confirmed linearity in the range 2–60 s. Thus,

TABLE III

KINETIC PARAMETERS FOR TRANSPORT AND UPTAKE OF 2-DEOXYGLUCOSE

Transport (zero-trans) was performed using 1.5–5 s incubations with 2-deoxyglucose at the concentrations shown for 3-*O*-methylglucose (3*O*MG) in Fig. 2B. Uptake experiments refer to 8 min incubations with 2-deoxyglucose at various concentrations as shown in Fig. 5, inset. K_i is the inhibition constant of 3-*O*-methylglucose on 8 min uptake of 5 μM 2-deoxyglucose. 3-*O*-Methylglucose was added 10 min before the labelled 2-deoxyglucose and was therefore present at equal concentrations on the two sides of the cell membrane. Mean ± 1 S.D. of four experiments in each group.

	Transport		Uptake		3*O*MG inhibition of uptake K_i (mM)
	K_m (mM)	V_{max} (amol·s^{-1}·cell^{-1})	$K_{m,app}$ (mM)	$V_{max,app}$ (amol·s^{-1}·cell^{-1})	
No treatment	1.2 ± 0.3	5.6 ± 1.0	0.51 ± 0.11	2.9 ± 0.3	3.9 ± 0.4
20 nM fMet-Leu-Phe	1.1 ± 0.3	34.8 ± 4.5	0.09 ± 0.07	3.8 ± 0.8	2.6 ± 0.3

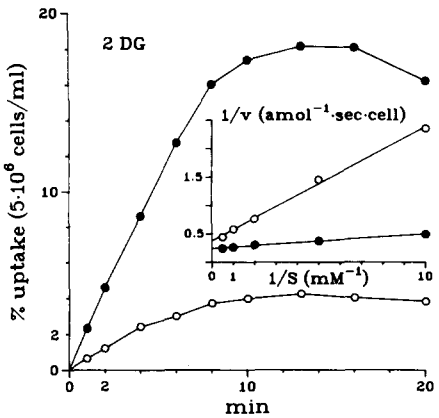

Fig. 5. Uptake of 2-deoxyglucose. The main graph shows uptake of 5 μM labelled 2-deoxyglucose in untreated and fMet-Leu-Phe-treated cells. The inset shows the concentration dependence of 2-deoxyglucose uptake 0–8 min. The calculated apparent K_m and V_{max} values are for untreated (○———○) $K_{m,app} = 0.65$ mM, $V_{max,app} = 2.7$ amol·s^{-1}·cell^{-1} and for fMet-Leu-Phe-treated cells (●———●) $K_{m,app} = 0.11$ mM, $V_{max,app} = 4.2$ amol·s^{-1}·cell^{-1}.

uptake of 2-deoxyglucose at a very low concentration is determined by the transport step. The inset shows the concentration dependence of uptake (8 min) and the apparent K_m value in fMet-Leu-Phe-treated cells is about 0.1 mM, as summarised in Table III. The very low apparent K_m values for the stimulated cells show that the rate-limiting step for uptake of the metabolizable sugars shift from transport at very low substract concentrations to metabolism (probably phosphorylation) at about 0.1 mM 2-deoxyglucose. Similar experiments measuring the inhibition of ^{14}C-labelled 2-deoxyglucose uptake by the non-metabolizable but equally well transported 3-*O*-methylglucose should serve as a control and yield inhibition constants much higher than the apparent K_m for 2-deoxyglucose uptake. Table III shows that this is the case.

Discussion

Glucose transport in polymorphonuclear leucocytes was previously evaluated using labelled 2-deoxyglucose as a probe and the stimulators used in the present study were reported to increase uptake [1–4]. It was shown that uptake was markedly inhibited by cytochalasin B at a saturating concentration, indicating that 2-deoxyglucose is transported across the cell membrane via the glucose transporter [2]. However, since the incubations were carried out for 15–30 min, uptake was a measure of phosporylation rather than transport. Here we show that the rate of transport actually equals the rate of phosphorylation for several min at a tracer concentration of 2-deoxyglucose both in resting and stimulated cells (Fig. 5). Thus, 2-deoxyglucose is in fact a faithful probe under this condition. However, uptake measurements yield kinetic constants markedly different from those obtained in transport experiments. For instance, in fMet-Leu-Phe-treated cells, K_m for uptake is around 0.1 mM (Fig. 5 and Ref. 1) whereas K_m for transport is around 1 mM. This shows that transport ceases to be rate-determining for uptake at 2-deoxyglucose concentrations higher than about 0.1 mM, so that the operational K_m for uptake becomes similar to K_m for hexokinase. Another point is that 2-deoxyglucose uptake, but not transport, is dependent on ATP. Therefore, uptake of 2-deoxyglucose, even at a tracer concentration, will not be a measure of transport if the cells, for instance in studies using inhibitors, are depleted of ATP.

We recently described that 3-*O*-methylglucose, in contrast to previous reports [4,12], is transferred by facilitated diffusion across the membrane of the resting leucocyte [5]. Here we show that K_m for zero-trans entry (all sugar analogue initially outside the cell) is around 1 mM and that fMet-Leu-Phe increases V_{max}. This K_m, which is similar for glucose and 2-deoxyglucose, is lower than in most other cell types (cf. Ref. 6). This may be related to the functions of leucocytes at extravascular locations with low glucose concentrations. In resting, but not stimulated, cells intracellular 3-*O*-methylglucose (exchange conditions) increased transport K_m 3–4 fold. Such phenomenon has been noted in several cell types [6], although its possible functional significance remains unknown.

The marked increase in the distribution volume for 3-*O*-methylglucose upon stimulation to our knowledge has not been described in any other cell type. A small part of the increase is explained by fMet-Leu-Phe-induced activation of Na^+–H^+ exchange which leads to cell swelling as previously detected by electronic sizing [13]. However, most of the increase is not explained by the increase in intracellular water volume. We do not know how

intracellular compartment(s) become accessible to 3-*O*-methylglucose upon stimulation, but it may be hypothesised that vesicles containing granula are impermeable to sugar although, of course, permeable to ^3H_2O. When the vesicles extrude their content after fMet-Leu-Phe stimulation, they fuse with the cell membrane and now embrace the compartment accessible to sugar. In addition, treatment with phorbol ester causes the rapid formation of large vesicles which are likely to be permeable to sugar since they are probably derived from the plasmalemma [14]. The concomitant approx. 5-fold increase in V_{max} for zero-trans entry would imply either a higher turnover of sugar on each transporter or an increase in the number of functional transporters in the cell membrane. The latter mechanism seems to be the most important in the well-characterised insulin-stimulated transport system of adipocytes [15–17].

The polymorphonuclear leucocyte depends on glucose as a source of energy for motility and chemotaxis. It has been reported that stimuli of chemotaxis, including fMet-Leu-Phe and C5a, do not activate glycogen phosphorylase, whereas phagocytosis does. This lead to the proposal that the increased entry of extracellular glucose was particularly important for providing energy for chemotaxis [3]. However, the present demonstration that thrombin does not augment glucose transport, even though it is as effective as fMet-Leu-Phe in chemotaxis [9], shows that stimulation of the transport system is not essential for this process.

The fMet-Leu-Phe-induced release of arachidonic acid is supposed to be a critical prerequisite for the stimulation of glucose transport [1,3]. Thrombin is effective in releasing arachidonic acid in smooth muscle cells [18] and peritoneal macrophages [19] and this may also occur in the leucocytes. If so, it seems interesting to explore the possible changes in metabolism of arachidonic acid caused by fMet-Leu-Phe to induce transport stimulation or by thrombin to prevent it.

In summary, we have shown that some chemotactic factors (e.g., fMet-Leu-Phe) but not others (thrombin) enhance 3-*O*-methylglucose, 2-deoxyglucose and glucose transport approx. 5-fold. When sugar is initially only present extracellularly, K_m is about 1 mM and an increase in V_{max} seems to account for the stimulation of transport. The transport of 2-deoxyglucose is rate-determining for its phosphorylation only at concentrations lower than about 0.1 mM.

Acknowledgements

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