Mechanisms of basal and cytokine-induced uptake of glucose in normal human eosinophils: relation to apoptosis

Per Venge\textsuperscript{a,}, Lena Moberg\textsuperscript{a,}, Eythor Björnsson\textsuperscript{b,}, Mats Bergström\textsuperscript{c,}, Bengt Långström\textsuperscript{c,}, Lena Håkansson\textsuperscript{a}

\textsuperscript{a}Department of Medical Sciences, Clinical Chemistry, University of Uppsala, Uppsala, Sweden
\textsuperscript{b}Respiratory Medicine and Allergology, University of Uppsala, Uppsala, Sweden
\textsuperscript{c}PET-centrum, University of Uppsala, Uppsala, Sweden

Summary A link between glucose transport and apoptosis was suggested. We studied the mechanisms of glucose transport in human eosinophils by means of the uptake of the positron emitting analogue, \textsuperscript{18}Fluoro-2-Deoxyglucose (FDG) and apoptosis by means of flow cytometry. FDG uptake was inhibited by antibodies to GLUT1, 3 and 4 and by cytochalasin B. The anti-apoptotic principles IL-5, GM-CSF, IL-3 enhanced the uptake, whereas the apoptosis-inducing principles anti-CD95 (anti-Fas) and exposure to serum-coated Sephadex particles caused a reduction. Also TNF-\textalpha enhanced the uptake. Other cytokines such as IL-2, IL-4, IL-8, RANTES and MCP-3 had no effect on the glucose uptake. 2-Deoxyglucose, antibodies to GLUT4 and CD95 induced apoptosis. The basal FDG-uptake was unaffected by PKC inhibitors Ro-31-8220, Go-6983 and Go-6976, whereas the latter inhibited the IL-5-enhanced uptake possibly due to the inhibition of PKC\textsubscript{m}. Protein tyrosine kinase and PI-3 kinase inhibitors inhibited IL-5-enhanced FDG-uptake only. In contrast MEK inhibitors inhibited the basal uptake only. Inhibitors of p38 MAP kinase inhibited both basal and IL-5 enhanced uptake. We conclude that glucose uptake in eosinophils is governed by specific intracellular mechanisms involving mobilization of GLUTs, Ca\textsuperscript{2+} and the activation of the MAP kinase pathway and that the IL-5-enhanced uptake uniquely seems to involve PKC\textsubscript{m} activity. Our results suggest a close link between apoptosis and glucose transport in human eosinophils.

KEYWORDS Glucose transport; GLUT; Eosinophil; Signal transduction; Apoptosis

Introduction

The eosinophil granulocyte is an inflammatory cell with potent biological capabilities of which the cytotoxic activities are the most conspicuous.\textsuperscript{1} The cytotoxic potential of eosinophils is related to the production and secretion of a number of very basic and cytotoxic proteins such as eosinophil cationic protein (ECP) and major basic protein (MBP), but also to the fact that the eosinophil is a very potent producer of reactive oxygen species. It is generally believed that the eosinophil is involved in parasite killing, but possibly also in the killing of tumour cells. Eosinophils are also involved in a number of inflammatory diseases of which the best known is bronchial asthma.

The attraction of eosinophils to sites of inflammation and the subsequent activation of eosinophils at the site involves a complex network of...
different molecules and cellular interactions. However, some molecules seem to stand out as particularly important and these are the cytokines and chemokines. Thus, the cytokine interleukin-5 (IL-5) is of great importance in growth and maturation of eosinophils as well as in the priming and activation of mature cells. One example of the importance of IL-5 are the experiments in which antibody-inhibition of the activity of IL-5 in broncho-alveolar lavage fluid obtained from allergic asthmatics completely abrogated the capacity of the fluid to attract eosinophils, although other chemotactic principles were present in the fluid as well. Another potentially very important effect of IL-5 is anti-apoptosis. This effect of IL-5 is shared with GM-CSF and IL-3 and is believed to contribute to the accumulation of eosinophils in certain inflammatory processes.

Recent data have suggested a relationship between apoptosis and glucose uptake. In one study on a myeloid cell line, IL-3 was shown to increase the uptake of glucose, and the induction of apoptosis by interaction with the Fas-receptor resulted in a prompt reduction in glucose uptake. Also in other cellular systems glucose uptake and apoptosis seem to be related and have common signal transduction pathways such as protein tyrosine kinase activation. The aim of this study was to investigate the mechanisms involved in glucose transport in eosinophil granulocytes, since such information is completely lacking and since such knowledge might provide us with further insight into the mechanisms involved in apoptotic and anti-apoptotic mechanisms in these cells. For this purpose we have studied the uptake of the glucose analogue, 18F-fluoro-2-Deoxyglucose (FDG), which is a positron emitting compound. The advantage of using this positron-emitting compound for these purposes is the small number of cells needed for each experiment. It is also of importance to understand the biochemical mechanisms governing the uptake of FDG in inflammatory cells, since this radiotracer is increasingly been used in the diagnosis of cancer and inflammatory processes.

Material and methods

BAPTA/AM, G6-6983, Gö-6976, Ro-31-8280, Genistein, Wortmannin, Calyculin A, PD 98059, Ly294002, SB 203580 were all purchased from Calbiochem. Antibodies to GLUT1-5 were purchased from Alpha Diagnostic International (USA). Anti-CD95 (clone CH-11) was from Immunotech (France). Dexamethasone and methyl prednisolone were from Pharmacia Corporation (USA).

The cytokines IL-2, IL-3, IL-4, IL-5, IL-8, GM-CSF, RANTES, MCP-3, TNF-α were all purchased from R&D (USA).

Serum-coated Sephadex-particles were prepared as described previously. 18FDG was produced using the GE PETtrace FDG MicroLab unit from GEMS PET Systems, which is an automated radiochemistry system for the production of 2-[18F]-fluoro-2-Deoxyglucose from [18F]-fluoride utilizing the process described by Toorongian et al.

Eosinophils were isolated from the blood healthy individuals as previously described. Briefly, mononuclear leukocytes were isolated by separation using Percoll gradient centrifugation and the eosinophils were separated from the neutrophils by negative immunoselection using anti-CD16 coated particles (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Neutrophils were purified by positive immunoselection using anti-CD16 coated particles. The purity of both cell types was >98%. The uptake studies of 18FDG in eosinophils or neutrophils was performed as follows: 100 µl of a cell suspension containing $10^6$ cells/ml was mixed with 25 µl of 18FDG (final concentration on average 0.2 µmol/l) in the presence of a final concentration of glucose of 0.2 mmol/l. After this 100 µl phosphate buffered saline was added containing stimulators and/or inhibitors at indicated concentrations. The final incubation volume was 225 µl containing 50,000 cells. The mixture was allowed to incubate for 40 min in most experiments after which the cells were washed by centrifugation 6 times with 2 ml of PBS each time. When enhancers and inhibitors were used these were co-incubated with the mixture for 40 min unless otherwise indicated in the results section. The radioactivity in the cell pellet was subsequently counted in a gamma counter. Because of the rapid decay of 18FDG a reference was counted in parallel and the counts adjusted automatically.

Apoptosis of eosinophils was evaluated by flow cytometry. The eosinophils, $1 \times 10^7$/l, were cultured in 96-well tissue culture plates in a medium consisting of RPMI-1640 medium (Life Technologies, Paisley, Scotland), 20% FCS (Life Technologies), L-glutamine (2 mmol/l) (Biochrom KG, Berlin, Germany), penicillin (100 IU/ml) and streptomycin (100 µg/ml) (Biochrom KG) in 5% CO2 at 37 °C. The cells were co-cultured with 2-deoxyglucose (0.5–20 mmol/l) and with antibodies to GLUT1, 2, 3 and 4 (5 µg/ml) and CD95 (2 µg/ml), respectively, for 24 h. After culture, the eosinophils were aliquoted into tubes and incubated for 30 min on ice with FDA.
(0.17 mg/l) (Sigma-Aldrich, St. Louis, MI, USA) or Annexin V (PharMingen, San Diego, CA, USA). Then, the cells were washed with PBS containing 0.5% (w/v) BSA and analysed by flow cytometry. The flow cytometer used was a Coulter EPICS XL-MCL (Coulter Company Inc., Miami, FL, USA). Instrument calibration was performed daily according to the recommendation of the manufacturer. On average, 10,000 cells from each sample were analysed in the flow cytometer. The relative number of incubated for 30 min on ice with FDA (0.17 mg/l) (Sigma-Aldrich, St. Louis, MI, USA) or Annexin V (PharMingen, San Diego, CA, USA). Then, the cells were washed with PBS containing 0.5% (w/v) BSA and analysed by flow cytometry. The flow cytometer used was a Coulter EPICS XL-MCL (Coulter Company Inc., Miami, FL, USA). Instrument calibration was performed daily according to the recommendation of the manufacturer. On average, 10,000 cells from each sample were analysed in the flow cytometer. The relative number of incubated for 30 min on ice with FDA

Results

Basal and cytokine-induced uptake of FDG

FDG is spontaneously taken up by the eosinophil and the uptake increased linearly with time as is shown in Fig. 1a. The effects of increasing concentrations of glucose in the medium are shown in Fig. 1b. At the concentration of 0.6 mM glucose the uptake was inhibited by 50% (IC50). For comparison the basal uptake of FDG in normal human neutrophils is also shown and was in most experiments two to three times as high as the uptake in eosinophils. Also the inhibitory effect of external glucose on neutrophil FDG-uptake is shown. The IC50 on the neutrophil uptake was about 0.2 mM glucose. These data suggest differences in the glucose transport mechanisms between eosinophils and neutrophils.

The incubation of eosinophils with the growth factors IL-5, GM-CSF or IL-3 resulted in a dose dependent enhancement of the FDG-uptake (Fig. 2), whereas the effects of a number of other cytokines were absent, such as for IL-2, IL-4, IL-8, RANTES and MCP-3. In most experiments the enhancement of the FDG-uptake was the highest for IL-5 followed by GM-CSF, whereas the uptake induced by IL-3 was increased, but at a lower level. Maximal enhancement with IL-5 was achieved at concentrations of 500 ng/10^6 eosinophils and no further enhancement was achieved by the addition of GM-CSF at maximal concentrations (data not shown). Nor did preincubation with IL-5 at concentrations of 5 ng/10^6 eosinophils for 30 min at 37°C result in any stimulatory effects of any of the above-mentioned cytokines. Also the incubation with TNF-α caused a dose related enhancement with optimal concentrations at 50 ng/10^6 eosinophils (Fig. 2). For comparison we incubated eosinophils with a strong secretagogue, serum-coated Sephadex particles. This incubation resulted in a dose related strong inhibition of the basal FDG-uptake (Fig. 3).
Glucose transporters in human eosinophils

In Table 1 the effects of cytochalasin B on basal and IL-5-enhanced FDG-uptake is shown. The results show that Cytochalasin B almost completely prevented any uptake of FDG. This suggests the involvement of GLUTs in the transporter process, since Cytochalasin B was shown to be a potent blocker of GLUTs. In order to study the role of GLUTs in the transport and uptake of FDG into the eosinophils further, we used monoclonal antibodies against GLUT1–5 to block the activity of the GLUT molecules. The results are shown in Fig. 4. Antibodies against GLUT 1, 3 and 4 inhibited FDG uptake in a dose-dependent manner, with antibodies against GLUT4 being the most potent, whereas the effects of antibodies against GLUT 2 and 5 were minor or absent. This pattern was seen both with basal uptake of FDG as well as with IL-5 enhanced uptake (data not shown).

Induction of apoptosis

Eosinophils were cultured in the presence of increasing concentrations of the glucose analogue 2-Deoxyglucose. After 24 h of culture in the presence of >1 mmol/l 2-Deoxyglucose the proportion of FDA positive cells was progressively decreased indicating reduced viability, while the proportion of Annexin V positive cells progressively increased as a sign of apoptosis-induction (Fig. 5). The experiment confirms the notion that glucose deprivation induces apoptosis. In the next set of experiments eosinophils were cultured in the presence of various GLUT-antibodies and the relative amount of apoptotic eosinophils was measured after 24 h. Co-culture with antibodies to GLUT4 partially and markedly increased the relative number of Annexin V positive, apoptotic cells, whereas antibodies to GLUTs 1, 2 and 3 had no effect (Fig. 6). Furthermore, co-culture with anti-CD95, as might be expected, induced apoptosis and to a similar extent as anti-GLUT4. In parallel the proportion of FDA positive, viable, eosinophils was markedly reduced by co-culture with anti-GLUT4 and anti-CD95 (54% and 40% versus 71%), but not with anti-GLUT1, 2 and 3.

Modification of the FDG uptake by various inhibitors of intracellular signal transduction pathways

In order to study the intracellular transduction signals involved in both basal and IL-5 enhanced
FDG-uptake we made a survey using a number of inhibitors of key activities.

(a) The basal or IL-5 enhanced FDG uptake was not inhibited by EDTA (not shown), but by the intracellular Ca-chelator BAPTA/AM (Table 2), which indicates that intracellular changes in Ca\(^{++}\) are involved.

(b) To study the involvement of Protein kinase C (PKC) we incubated the eosinophils with various inhibitors such as Ro-31-8220, G\(\text{o}-6976\) and G\(\text{o}-6983\). The inhibitors were chosen to cover most of the known activities of the PKC isoenzyme (Calbiochem, Signal Transduction Catalog & Technical Resources, 1999, p. 305). Ro-31-8220 or G\(\text{o}-6983\) did not affect the basal nor the IL-5 enhanced FDG uptake at concentrations from \(10^{-6}\) to \(10^{-5}\) mol/l. G\(\text{o}-6976\) did not affect the basal FDG uptake, whereas the IL-5 enhanced uptake was inhibited in a dose dependent manner with a complete inhibition at \(10^{-5}\) mol/l (Fig. 7a). These data suggest that PKC activation is not involved in the basal uptake of FDG, whereas activation of PKC-\(\mu\) is suggested to be involved in the IL-5 enhanced FDG-uptake.\(^{19}\)

(c) The involvement of tyrosine kinase activity was studied by means of Genistin. Genistin inhibited the basal FDG uptake by about 25% but

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**Table 1** FDG-uptake by eosinophils and the effects of cytochalasin B.

<table>
<thead>
<tr>
<th></th>
<th>Basal FDG-uptake (%)</th>
<th>IL-5 enhanced FDG-uptake (%)</th>
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<tbody>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>10 µg/ml</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2 µg/ml</td>
<td>11.0</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
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Eosinophils were incubated for 40 min with cytochalasin B together with FDG and either buffer or IL-5 (500 ng/\(10^6\) eosinophils), after which the cells were washed and the uptake was measured. Results are presented as % of control, i.e. the basal FDG-uptake in buffer medium.
only at the highest concentration, i.e. \(10^{-5}\) mol/l. In contrast the IL-5 induced enhancement was inhibited in a dose dependent manner and completely at \(10^{-5} - 10^{-6}\) mol/l (Fig. 7b). The results suggest the involvement of protein tyrosine kinase activity in the IL-5 enhanced FDG-uptake.

(d) The two specific inhibitors of PI-3 kinase, Wortmannin and Ly 294002 did not affect the basal FDG uptake. The IL-5 enhanced uptake was inhibited in a dose dependent manner with an almost complete inhibition at \(10^{-6} - 10^{-7}\) mol/l (Fig. 7c and Table 2), suggesting the involvement of PI-3 kinase activity in this enhancement, but not in the basal uptake of FDG.

(e) The MEK inhibitor PD 098059 inhibited the basal FDG uptake by about 25% at \(10^{-5}\) mol/l. The IL-5 enhanced uptake (Table 2) was inhibited to the same extent and was likely attributed to the inhibition of the basal FDG uptake. Thus an inhibitory activity specifically directed towards the IL-5 enhanced part of the uptake was not suggested by the experiments. In contrast the specific inhibitor of the p38 MAPkinase, i.e. SB 203580, was a potent inhibitor of both basal and IL-5-enhanced glucose uptake (Fig. 7d). Our results therefore suggest that MEK is involved in the basal uptake of FDG only, whereas the p38 MAPkinase may

Table 2  FDG-uptake by eosinophils and the effects of various inhibitors of intracellular transduction pathways.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Basal FDG-uptake (%)</th>
<th>IL-5 enhanced FDG-uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>BAPTA/AM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2.5 \times 10^{-5})</td>
<td>18</td>
<td>4.3</td>
</tr>
<tr>
<td>(10^{-5})</td>
<td>80</td>
<td>6.6</td>
</tr>
<tr>
<td>(10^{-6})</td>
<td>125</td>
<td>109</td>
</tr>
<tr>
<td>(10^{-7})</td>
<td>127</td>
<td>na</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>LY294002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^{-5})</td>
<td>85</td>
<td>95</td>
</tr>
<tr>
<td>(10^{-6})</td>
<td>88</td>
<td>83</td>
</tr>
<tr>
<td>(10^{-7})</td>
<td>108</td>
<td>85</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PD098059</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^{-5})</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>(10^{-6})</td>
<td>118</td>
<td>109</td>
</tr>
<tr>
<td>(10^{-7})</td>
<td>97</td>
<td>105</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Calyculin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10^{-7})</td>
<td>47</td>
<td>na</td>
</tr>
<tr>
<td>(10^{-8})</td>
<td>63</td>
<td>34 (2 ×)</td>
</tr>
<tr>
<td>(10^{-9})</td>
<td>89</td>
<td>93 (2 ×)</td>
</tr>
<tr>
<td>(10^{-10})</td>
<td>109</td>
<td>98 (2 ×)</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100 (2 ×)</td>
</tr>
</tbody>
</table>

The respective inhibitor was incubated together with FDG and buffer (basal uptake) or IL-5 (500 ng/10⁶ eosinophils) (IL-5 enhanced uptake) for 40 min after which the cells were washed and radioactivity in the pellet measured. Results are presented as % of control, i.e. the basal FDG-uptake in buffer medium. na = not applicable; (2 ×) indicates that these experiments were performed with two times higher concentrations of calyculin A.
be involved in both the basal and IL-5 enhanced uptake.

(f) The phosphatase inhibitor Calyculin A potently inhibited both the basal and the IL-5 enhanced FDG uptake at concentrations from $10^{-8}$ to $10^{-5}$ mol/l (Table 2) confirming the importance of phosphorylation processes in the glucose transporter processes.

Effects of other inhibitors

Since a relationship between apoptosis induction and glucose transport has been suggested and further suggested above, we studied the effects of two mechanisms possibly involved. One is the interaction with the Fas-receptor and the other is the effect of corticosteroids.

Preincubation of eosinophils with monoclonal antibodies to the Fas-receptor, i.e. anti-CD95, for 120 min caused a dose dependent reduction in the basal FDG uptake (Fig. 8). Pre-incubation of the cells for 50 min with either Dexamethason in the concentration range from $10^{-7}$ to $10^{-9}$ mol/l or Methylprednisolone in the concentration range from $10^{-6}$ to $10^{-8}$ did not affect either the basal FDG uptake nor the IL-5 enhanced uptake (data not shown). These data indicate that induction of apoptosis by anti-Fas reduces the uptake of FDG, whereas no effects of corticosteroids were discernible.
Our results have shown that $^{18}$FDG may be used as a substitute for glucose in the study of glucose transporter mechanisms in eosinophils and neutrophils, since the uptake of $^{18}$FDG was clearly dependent on glucose transporter molecules, i.e., GLUT, and since glucose competed with the uptake of $^{18}$FDG. This conclusion is in agreement with that of others using other cell systems and the use of $^{18}$FDG as an in vivo tool in the detection of metabolically active tissues. One advantage of using FDG as a tool to study glucose uptake was the minimum requirement of cells, in particular when one is studying purified eosinophils. Although we used 50 000 cells as standard in our incubations, the use of less than one-tenth of this number, i.e., to less than 5000 cells per incubation, would have been possible. It was suggested by our results that eosinophils and neutrophils use partly different mechanisms for the basal uptake of $^{18}$FDG and that the affinity of $^{18}$FDG to the eosinophil glucose transporter mechanisms was about three times higher than for the neutrophils. On the other hand the basal uptake of $^{18}$FDG in neutrophils was consistently higher than that seen for normal eosinophils. Earlier reports showed a similar rate of metabolism of $^{14}$C-glucose for normal eosinophils and neutrophils, whereas the rate was much higher in eosinophils obtained from patients with eosinophilia. Also the uptake of 2-Deoxyglucose was much higher in such eosinophils, which may be compatible with our results on growth factor stimulated $^{18}$FDG uptake. It should be emphasized that the uptake of $^{18}$FDG only reflects the actual transport of glucose across the plasma membrane and not glucose metabolism, since $^{18}$FDG is not metabolised further, but bound irreversibly to hexokinase. Therefore, the comparison of results of uptake and those of actual metabolism of glucose is difficult. The dependence on glucose transporters for the uptake of $^{18}$FDG was suggested by the potent inhibition by cytochalasin B of both the basal and the IL-5 enhanced uptake, but also by the inhibition of glucose uptake by antibodies to the various GLUTs. The fact that antibodies to either GLUT 1, 3 or 4 at the highest concentrations almost completely inhibited the $^{18}$FDG uptake suggested that these molecules cooperate or that these three glucose transporter molecules share common epitopes involved in the glucose transport. It should be emphasized that the inhibitory effects of the three GLUT antibodies were similar whether basal glucose or IL-5 enhanced glucose uptake was studied, indicating that these molecules are operative in both situations. The presence of GLUT4 may be surprising, since this is the GLUT molecule normally regulated by insulin and since it is assumed that glucose uptake by eosinophils is independent of insulin. In preliminary studies we could not find any effect of insulin on $^{18}$FDG uptake in eosinophils supporting this notion. The absent effect of GLUT5 may be explained by the fact that this transporter molecule is operative preferentially in fructose transport and the absent effect of GLUT2 by the fact that it has been shown to have a much lower affinity for glucose than the other GLUTs. In preliminary flow cytometry studies, though, all five GLUTs were found to be present in eosinophils when saponin was used to permeabilize the cells.

The enhancement of glucose uptake by IL-3 and GM-CSF has been shown in neutrophils, T-lymphocytes and some cell lines, whereas the effects on eosinophils have not been documented. We found that IL-5 in addition to those two growth factors and in addition to TNF-$\alpha$ uniquely enhanced glucose uptake, since other cytokines (e.g., RANTES, MCP-3), with agonist effects on eosinophils, had no effect. This implies that the glucose transporter mechanisms are related to growth, survival and priming of eosinophils rather than to effector functions such as secretion, migration, etc., although glucose is the major energy source for eosinophils.
substrate for such activities. It is therefore of interest that molecules with opposite actions to these growth factors, i.e. principles, which induce apoptosis, such as anti-CD95, at the same time reduce glucose transport. Moreover, a classical stimulus for degranulation, i.e. complement coated particles, actually reduced the basal $^{18}$FDG-uptake. This latter finding may be an example of the induction of apoptosis, since earlier workers showed that induction of eosinophil degranulation induced signs of apoptosis in the cells. Since other studies have indicated an apoptosis inducing effect of glucocorticosteroids on eosinophils, we expected to find an adverse effect of these compounds on the $^{18}$FDG-uptake. Our failure of showing such an adverse effect of glucocorticosteroids, however, may be due to the short-term incubations. For unknown reasons long-term experiments with incubations of eosinophils with glucocorticosteroids, i.e. for 20 h, showed very inconsistent results and any conclusions from these were not possible to draw. Thus, any effect of glucocorticosteroids on the $^{18}$FDG-uptake cannot be entirely excluded.

The link between glucose uptake in eosinophils and apoptosis was indeed suggested by our findings that glucose deprivation by the incubation of the eosinophils with the competitor molecule 2-Deoxyglucose potently induced apoptosis. The dependence on glucose uptake was also indicated by the inhibitory effects of antibodies against GLUT4. Our findings on eosinophils, therefore, parallel the findings in several other cellular systems. Surprisingly, though the other GLUT-antibodies were without effect, although they inhibited glucose uptake as noted above. Differences in potencies when it comes to the induction of apoptosis, however, maybe a matter of quantitative differences rather than qualitative differences, since the GLUT4 antibodies consistently were more potent in the inhibition of glucose uptake.

The signal transduction mechanisms involved in basal and IL-5 enhanced uptake were studied by the use of a range of inhibitors of key activities. Our results showed the pivotal role of intracellular calcium for both the spontaneous and IL-5 enhanced uptake, whereas depletion of extra cellular calcium was without any effect. Earlier studies have established that the expression of mRNA for GLUT1 in liver and muscle cells and the mobilization of the protein to the cell membrane are dependent on intracellular calcium. However, no information is available as to the dependence of calcium for glucose transport into eosinophils or other myeloid cells. It is interesting though, that chelation of intracellular calcium promoted apoptosis of neutrophils, thus emphasizing the likely link between glucose transport and apoptosis.

The results obtained in this study clearly indicated that the spontaneous, basal uptake of glucose from the environment and the growth factor enhanced uptake are governed by different intracellular signals. Thus, the effects of various putative inhibitors of protein kinase C indicated that the basal glucose uptake is independent of these pathways in the meaning that no inhibitions were seen with any of the inhibitors used. The IL-5 enhanced uptake was also unaffected by most PKC inhibitors, with the exception of the inhibitor Gö-6976. Gö-6976 is a general inhibitor of Ca$^{2+}$-dependent PKCs, but as opposed to the other general inhibitors, it uniquely inhibits the $\mu$- isoform of PKC. Consequently, our interpretation is that PKC$\mu$ may be involved in growth factor enhanced uptake of glucose. A similar observation was made in a murine mast cell model of allergen-stimulated cells, in which the effects of IL-5 and TNF-$\alpha$ were shown to be dependent on PKC$\alpha$. PKC$\mu$ was also suggested to be involved in IL-6-dependent proliferation of murine plasmacytoma cells. This novel finding in human eosinophils may therefore be an interesting target for future drug development.

The involvement of tyrosine kinase and PI-3 kinase activities in IL-5 enhanced, but not in basal glucose uptake, was suggested by the results in this study. These findings are compatible with earlier observations that IL-5 priming of eosinophils includes such activities. Also, the findings that the increased transporter affinity for glucose in other myeloid cells, as promoted by IL-3, was reduced by such inhibitors support our findings. The relationship between glucose uptake and apoptosis is further suggested by these experiments, since inhibitors of PI-3 kinase prevented glucose uptake and induced apoptosis. Earlier work, however, showed a relationship between Fas-induced apoptosis and MAPkinase activity in the human leukemic T cell line, Jurkat with a reduction in apoptotic cells after incubation with PD 098059, whereas in our experiments PD 098059 seemed to have little effect on IL-5 enhanced glucose uptake, but inhibited the basal uptake. The explanation to these seeming discrepancies could be due to the fact that different mechanisms are operative in different cells, although others have shown the dependence of MAPkinase activity for IL-5 and GM-CSF activation of eosinophils. Indeed we showed in this report that the p38 MAPkinase seems to be involved in IL-5 enhanced glucose uptake.

Which might be the actual link between reduced glucose transport and apoptosis in human
eosinophils? Triggering of the mitochondrial death cascade by intracellular depletion of ATP is one common mechanism in most other cells. Eosinophils, however, are almost lacking mitochondria and any oxidative phosphorylation does not take place in this cell. These data would imply that the involvement of mitochondria in apoptosis induction in eosinophils is less likely. However, recent studies have suggested that the few remaining mitochondria in eosinophils actually have preserved their apoptotic function, but not their respiratory function. Thus, lowering of intracellular ATP as a consequence of reduced glucose transport and glycolysis seems to be a reasonable hypothesis as to the mechanism of the induction of apoptosis also in human eosinophils.

We conclude that glucose uptake in eosinophils is dependent on the glucose transporter molecules 1, 3 and 4, and that molecules which are anti-apoptotic enhance this uptake and that apoptotic-inducing principles such as anti-Fas may reduce this uptake. We also confirm that, like in other cellular systems, blockage of glucose uptake induces apoptosis of eosinophils. Glucose uptake in eosinophils seems to be dependent on mobilization of intracellular Ca²⁺ and the MAP kinase pathway. The IL-5-enhanced uptake is initiated through mechanisms involving protein tyrosine kinase activity, PI-3 kinase and PKC. A close link between apoptosis and glucose transport in human eosinophils is suggested.

Acknowledgements

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References