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Inhibition of insulin-stimulated glucose transport in rat adipocytes by nucleoside transport inhibitors

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In isolated rat adipocytes, basal as well as insulin-stimulated 3-*O*-methylglucose transport was inhibited nearly completely (maximal inhibition: 95%) by the nucleoside transport inhibitors dipyridamole ($IC_{50} = 5$ μM), nitrobenzylthioguanosine (20 μM), nitrobenzylthioinosine (35 μM) and papaverine (130 μM). Transport kinetics in the presence of 10 μM dipyridamole revealed a significant increase in the transport K_m value of 3-*O*-methylglucose (3.45 ± 0.6 vs 2.36 ± 0.29 mM in the controls) as well as a decrease in the V_{max} value (4.84 ± 0.95 vs 9.03 ± 1.19 pmol/s per μl lipid in the controls). Half-maximally inhibiting concentrations of dipyridamole were one order of magnitude higher than those inhibiting nucleoside (thymidine) uptake (0.48 μM). The inhibitory effect of dipyridamole (5 μM) reached its maximum within 30 s. The agent failed to affect insulin's half-maximally stimulating concentration (0.075 nM) indicating that it did not interfere with the mechanism by which insulin stimulates glucose transport. Further, dipyridamole fully suppressed the glucose-inhibitable cytochalasin B binding ($IC_{50} = 1.65 \pm 0.05$ μM). The data indicate that nucleoside transport inhibitors reduce glucose transport by a direct interaction with the transporter or a closely related protein. It is suggested that glucose and nucleoside transporters share structural, and possibly functional, features.

Glucose transport; Nucleoside transport; Dipyridamole; (Adipocyte)

1. INTRODUCTION

Several lines of evidence have suggested that the carrier proteins mediating facilitated diffusion of glucose and nucleosides share functional and structural characteristics. In human erythrocytes, both transporters are band 4.5 membrane proteins [1,2]. Copurification of both transport systems has been reported in these cells: the purified preparations contained binding activities of both nitrobenzylthioinosine and cytochalasin B, inhibitors of nucleoside and glucose transport, respectively [1,3]. Further, reconstitution experiments revealed that the purified material contained stereospecific glucose transport activity as well as nucleoside transport activity [4,5]. In addition, attempts to label the nucleoside transporter with a photoactive derivative appeared to result in labeling of the glucose transporter [6].

In the present study, we tested the possibility that glucose and nucleoside transport can be differentiated with the aid of agents assumed to specifically inhibit the nucleoside transport. The results indicate that inhibitors of nucleoside transport are also potent inhibitors of glucose transport in rat adipose cells. The data suggest structural similarities, and possibly a functional relation, between the nucleoside transporter and the insulin-sensitive hexose transporter in adipose cells. Part of the results have been published in abstract form [7].

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2. MATERIALS AND METHODS

2.1. *Materials*

3-*O*-[^{3}H]Methylglucose, [^{3}H]thymidine and [^{3}H]cytochalasin B were purchased from Amersham (Braunschweig, FRG). Dipyridamole was a gift from Dr Karl Thomae GmbH (Biberach, FRG). Nitrobenzylthioinosine (NBTI), nitroben-

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zylthioguanosine (NBTG) and papaverine were supplied by Sigma (Deisenhofen, FRG). Crude bacterial collagenase was obtained from Cooper Biochemical; bovine serum albumin (fraction V) was from Serva (Heidelberg, FRG).

2.2. *Glucose transport*

Adipose cells were prepared from epididymal fat pads of male Wistar rats according to the method of Rodbell [8] with minor modifications [9]. Cells were incubated in a Krebs-Ringer Hepes buffer at 37°C with the agents under investigation. Where indicated, a preincubation period of 20 min with insulin in a concentration of 0.8 nM was performed in order to produce maximal activation of glucose transport. Glucose transport activity was determined with the aid of the non-metabolizable hexose, 3-*O*-methylglucose as described [9]. Initial methylglucose uptake rates were calculated as described in [10]. All data were normalized per volume of lipid which was determined with the cytocrit method as described [11]. Cell count was determined in parallel samples, and 1 μl of lipid routinely contained 10000 cells.

2.3. *Nucleoside transport*

Thymidine uptake was determined at a substrate concentration of 10 μM, which is approximately one tenth of the transport K_m value in adipocytes (Steinfelder, unpublished). Isolated adipocytes at a cytocrit of 10–15% were incubated for 20–30 min prior to the assay. Uptake was initiated by the addition of [^{3}H]thymidine (final concentration 10 μM; 0.5 μCi), and the reaction was stopped after 15 s by the addition of 6.5 ml of a cold phloretin solution (0.3 mM, pH 7.4). Cells were separated from medium by centrifugation through silicone oil (density 0.96) within 3 min after termination of uptake. Equilibrium uptake was measured after incubation of the cells with the tritiated nucleoside for 1 h. After 15 s, about 30–40% of the equilibrium distribution space of the nucleoside were filled. The initial uptake rate V_i was calculated from the uptake rates U_t and U_{max} as described for methylglucose uptake in [10].

3. RESULTS

3.1. *Effect of nucleoside transport inhibitors on insulin-stimulated glucose transport*

Fig.1 illustrates the inhibitory effect of nucleoside transport inhibitors on the initial 3-*O*-methylglucose uptake rate in insulin-stimulated adipocytes. Isolated adipocytes were incubated with insulin (4.8 ng/ml) for 30 min, and the indicated concentrations of dipyridamole, papaverine, NBTI, or NBTG were added. After an additional incubation period of 5 min, methylglucose uptake was assayed as described in section 2. The agents inhibited the initial uptake rate of methylglucose in a concentration-dependent manner (fig.1). The maximal transport inhibition (approx. 95%) was very similar for all four agents, but significant differences in the IC_{50} values were

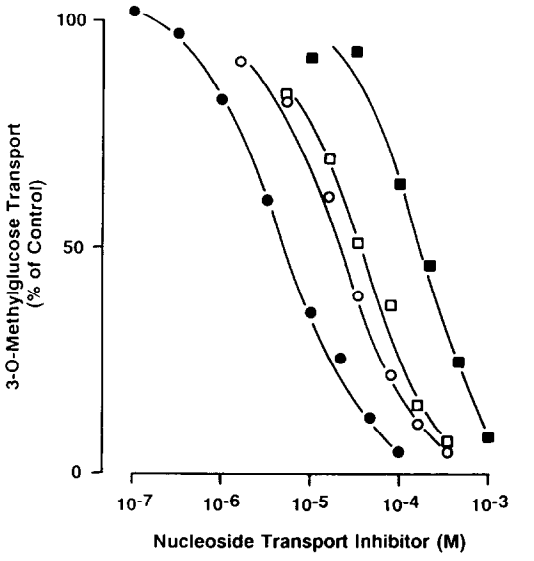

Fig.1. Inhibitory effect of nucleoside transport inhibitors on insulin-stimulated 3-*O*-methylglucose transport in isolated adipocytes. Dipyridamole (●), nitrobenzylthioinosine (□), nitrobenzylthioguanosine (○) and papaverine (■) were added 5 min prior to the transport assay. Data represent the means of 4 separate experiments. The mean control transport rate was 0.333 ± 0.015 pmol/s per μl lipid.

observed. The order of potency was: dipyridamole (IC_{50} = 5.1 ± 0.8 μM) > NBTG (21.4 ± 2.1 μM) > NBTI (36.4 ± 8.2 μM) ≫ papaverine (130 ± 13 μM). Since dipyridamole was the most potent inhibitor, all further experiments were performed with this agent.

Transport inhibition observed in the presence of dipyridamole (5 μM) was not dependent on the order of addition of insulin and the inhibitor: the magnitude of inhibition was the same when the agent was added after (5 or 15 min) or simultaneously with insulin (not shown). A study of the time course of the effect of dipyridamole (5 μM) revealed that the inhibition approached its maximum 30 s after addition of the agent (not shown).

Fig.2 illustrates a series of experiments in which the concentration of insulin was varied. Dipyridamole reduced the initial methylglucose uptake rate by 35–45% at all tested concentrations of insulin. However, the concentration of insulin necessary to produce a half-maximal stimulation of glucose transport was not affected by the presence of 5 μM dipyridamole [0.072 ± 0.018 nM (0.43 ± 0.11 ng/ml) vs 0.077 ± 0.008 nM (0.46 ±

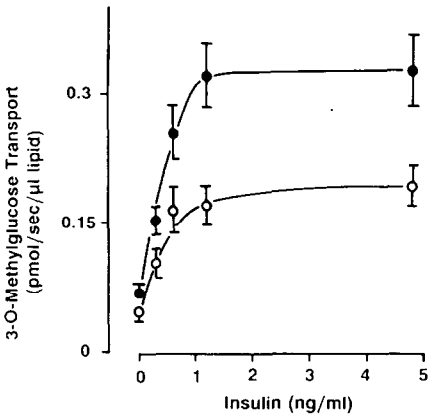

Fig.2. Concentration-dependent stimulation of glucose transport by insulin as measured in the presence (○) and absence (●) of dipyridamole (5 μM). The inhibitor was added together with the hormone at the start of the incubation. The transport assay was performed after 30 min of incubation at 37°C. Data represent the mean ± SE of 6 experiments.

0.05 ng/ml) in control cells]. Thus, dipyridamole failed to alter the sensitivity of the cells to insulin.

In a separate series of experiments the concentration dependence of the effect of dipyridamole was studied in basal adipocytes (not shown). The IC_{50} of the transport inhibitor was 7.1 ± 1.0 μM (n = 4) in basal cells as compared with 5.1 ± 0.8 μM in insulin-stimulated adipocytes (n = 4), indicating that insulin treatment of cells does not significantly alter the sensitivity of the cells to dipyridamole.

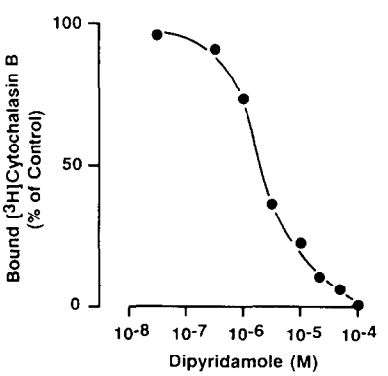

Fig.3. Concentration-dependent inhibition of the D-glucose inhibitable [^{3}H]cytochalasin B binding by dipyridamole in plasma membranes of insulin-stimulated adipocytes. IC_{50} = 1.65 ± 0.05 μM. Data are means of 4 experiments.

3.2. *Transport kinetics in the presence of dipyridamole (10 μM)*

In order to define the type of inhibition, the substrate dependence of insulin-stimulated glucose transport was studied in the presence and absence of dipyridamole (10 μM). V_{max} and K_m values obtained from Hanes plots are given in table 1. The data indicated that dipyridamole significantly affected the transport V_{max} value (45% inhibition) as well as the transport K_m value (45% increase). Therefore, dipyridamole appeared to inhibit glucose transport in an uncompetitive manner.

3.3. *Effect of dipyridamole on cytochalasin B binding*

Cytochalasin B is considered to be a specific ligand for the glucose transporter [2,12,13]. In an attempt to demonstrate a direct interaction of nucleoside transport inhibitors with the glucose transporter, the effect of dipyridamole on cytochalasin B binding was studied. Most non-specific binding sites were blocked with cytochalasin E as described [14], and the residual non-specific binding was assessed with samples incubated in the presence of 200 mM D-glucose. Fig.3 illustrates that dipyridamole fully displaced the glucose-inhibitable cytochalasin B binding in plasma membranes from insulin-treated adipocytes with an IC_{50} of 1.65 ± 0.05 μM.

3.4. *Effect of dipyridamole on nucleoside transport in rat adipocytes*

In order to compare the effects of dipyridamole

Table 1

Kinetic parameters of insulin-stimulated glucose transport in the absence and presence of dipyridamole (10 μM)

	V_{max} (pmol/s per μl lipid)	K_m (mM)
Control	9.03 ± 1.19	2.36 ± 0.29
Dipyridamole (10 μM)	4.84 ± 0.95	3.45 ± 0.60

Methylglucose uptake was assayed in cells pretreated with a maximally effective concentration of insulin at 5 different substrate concentrations. For kinetic evaluation the transport data were transformed in a Hanes plot, and values of V_{max} and K_m of the individual experiments were determined by linear regression. Data given are the means ± SE of 4 separate experiments

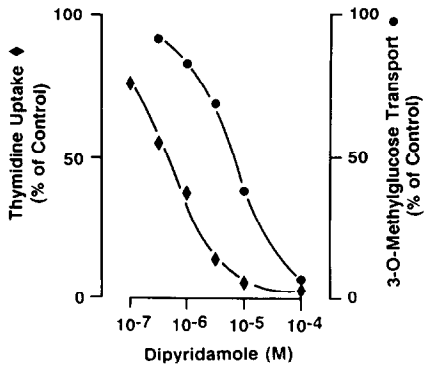

Fig.4. Comparison of the effects of dipyridamole on thymidine and 3-*O*-methylglucose transport in isolated adipocytes. Cells were incubated with 4.8 ng/ml insulin for 30 min prior to the transport assays. Data are the means of 4 experiments. Half-maximally inhibiting concentrations of dipyridamole were 0.48 ± 0.11 μM (thymidine uptake) and 5.25 ± 0.63 μM (glucose transport).

on glucose transport with those on nucleoside transport, [^{3}H]thymidine uptake was studied in isolated adipocytes. In preliminary experiments it was ascertained that thymidine uptake in adipocytes was a saturable process and that no metabolism of the nucleoside occurred (not shown). Thus thymidine appeared to be the most suitable substrate for the study of nucleoside transport in these cells. Fig.4 illustrates that the dipyridamole concentrations inhibiting uptake of the nucleoside (IC_{50} = 0.48 ± 0.11 μM) were about one order of magnitude lower than those reducing glucose transport in insulin-stimulated cells (IC_{50} = 5.25 ± 0.63 μM).

4. DISCUSSION

The present data indicate that nucleoside transport inhibitors also interfere with the insulin-dependent glucose transport in adipocytes. Our findings suggest that both transport systems share structural, and probably functional, characteristics. This conclusion is supported by the recent findings of Jarvis et al. [6] who showed that the adenosine derivative 8-azidoadenosine, which was initially used as a photoaffinity probe of the nucleoside transporter [15], appeared to label mainly the glucose transporter in human erythrocytes [6]. However, the present data indicate that the concentrations required for glucose transport inhibition were approximately one order of magnitude higher than those necessary for nucleoside transport inhibition. Thus, the site responsible for mediating the effect of the inhibitors may be structurally similar, but cannot be identical in both transporters. It should be noted, that nucleoside transport appears to be less sensitive to inhibitors in rat adipocytes than in human erythrocytes [1] and in S49 mouse lymphoma cells [16], because the IC_{50} values reported in these studies were lower than those observed here.

Three lines of evidence suggest that the agents interact directly with the transporter or a closely related protein. First, the nucleoside transport inhibitors appeared not to interfere with the mechanism by which insulin activates transport. Both basal and insulin-stimulated transport were inhibited, and the sensitivity of cells to insulin (EC_{50}) was unaffected by the agents. Second, the nucleoside transport inhibitor dipyridamole fully abolished the glucose-inhibitable component of cytochalasin B binding which is considered to reflect a binding site at the glucose transporter [2,12,14]. It has been reported that nucleosides interfered with the binding of cytochalasin B to plasma membranes of human erythrocytes [6]. Since cytochalasin B was a poor inhibitor of nucleoside transport in reconstitution experiments [5], and is therefore unlikely to bind to the nucleoside transporter, this finding suggested that nucleosides interact with the glucose transporter which is an abundant protein in erythrocyte membranes [17]. Third, the agents inhibited glucose transport within seconds after addition to the cells. This rapid onset of action is in marked contrast to the slower onset (half time 3–6 min) of the effects produced by catecholamines [18], ACTH [9] and insulin [19].

Kinetic experiments indicated an uncompetitive type of inhibition with alteration of both K_m and V_{max} values. Thus the nucleoside transport inhibitors do not appear to block the site that recognizes the glucose molecule. This mechanism has been suggested for the inhibitory effect of forskolin on glucose transport on the basis of data showing a competitive type of inhibition [10]. On the basis of the present data it cannot be excluded, therefore, that the binding site for the nucleoside transport inhibitors is not located at the glucose transporter but at a regulatory protein interacting with the transporter.

In summary, the glucose and the nucleoside transporter share a similar binding site through which transport is inhibited. Thus, the comparison of the two transport systems might allow elucidation of the molecular basis of their function.

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