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Acute stimulation of glucose transport by histamine in cardiac microvascular endothelial cells

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Abstract

The purpose of the present work was to study the acute regulation of glucose uptake in cultured cardiac endothelial cells (CEC). Two types of potential stimuli were considered: (1) agents that are known to acutely stimulate glucose transport (i.e., within minutes) in fat and muscle tissues and (2) agents that influence endothelial cell function. Among the former agents, neither insulin, nor catecholamines (adrenaline, dopamine, phenylephrine), nor serotonin affected the rate of glucose transport in CEC, while SH-group reagents (phenylarsine oxide, diamide or menadione) were inhibitory. Among the factors of the second group that were tested (heparin, ADP, histamine, bradykinin), histamine was found to stimulate glucose transport in CEC by 10–50%. This effect was concentration-dependent (with an EC_{50} value $\cong 12$ μM) and reached a maximum within 5 min upon histamine addition. This stimulation of glucose transport was suppressed by pyrilamine (100 nM), a specific H_1-receptor antagonist, but not by cimetidine (100 μM), a H_2-selective antagonist. Northern blot and Western blot analysis of CEC extracts revealed the presence of the ubiquitous glucose transporter isoform GLUT1 mRNA and protein, but not of the 'insulin-regulatable' isoform GLUT4. In conclusion, this is the first report on an acute stimulation of glucose transport in cardiac endothelial cells, in particular, and in an insulin-unresponsive cell type, in general. The effect of histamine is most likely mediated by H_1-receptors and cannot be accounted for by a recruitment of GLUT4.

Keywords: Glucose transport; Endothelial cell; Histamine; Insulin; GLUT1; GLUT4

1. Introduction

Glucose is the major substrate of energy metabolism in endothelial cells [1]. An increase in the rate of glucose uptake was reported to occur upon long-term treatments (i.e., over hours) such as glucose deprivation [2], hypoxia [3] or exposure to growth factors [4]. These effects are accounted for by an up-regulation of the glucose transporter isoform GLUT1 at the transcriptional level [2–4]. Although endothelial cells are subject to acute challenges, such as in the vascular responses to injury, infection or shear stress, which might also influence endothelial cell energy metabolism, knowledge about a rapid regulation of glucose transport and metabolism in these cells is very scarce. The possibility of such a short-term control was suggested on the basis of immunocytochemical investigations by Vilaro et al. [5]. These authors reported that endothelial cells of fat, muscle and heart tissues not only express the 'insulin–regulatable' glucose carrier isoform GLUT4, but also respond to an insulin administration in vivo with a translocation of GLUT4 to the plasma membrane [5]. In contrast, endothelial cells of liver and brain (tissues that do not respond to insulin with respect to glucose transport) do not contain GLUT4 [5]. In cultured endothelial cells, long-term [6–8], but not short-term [2,4] stimulatory effects of insulin on glucose transport were detected. Since the cells used in these latter studies were obtained from insulin-unresponsive tissues such as brain [2] or aorta [4], extrapolation of the findings to insulin-responsive organs such as the heart [9,10] should be done with precaution. We therefore examined the effect of

Abbreviations: dGlc, 2-deoxy-D-glucose; CEC, cardiac endothelial cells.

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insulin on glucose transport in cultured endothelial cells obtained from adult rat heart. Furthermore, we tested the action (1) of agents known to mimic the effect of insulin in adipocytes and myocytes, such as SH-reagents [11–15], catecholamines [9,16,17] or serotonin [16] and (2) of regulators of endothelial cell function, such as bradykinin [18–21], histamine [22–24], heparin [25,26] or adenosine diphosphate [27,28].

2. Materials and methods

2.1. Materials

2-Deoxy-D-[^{3}H]glucose was purchased from Amersham (Braunschweig, FRG); culture medium RPMI 1640, M 199, Penicillin, Streptomycin Gentamycin, Fungizon, trypsin (Gibco); collagenase (Cooper Biomedical, Wor-

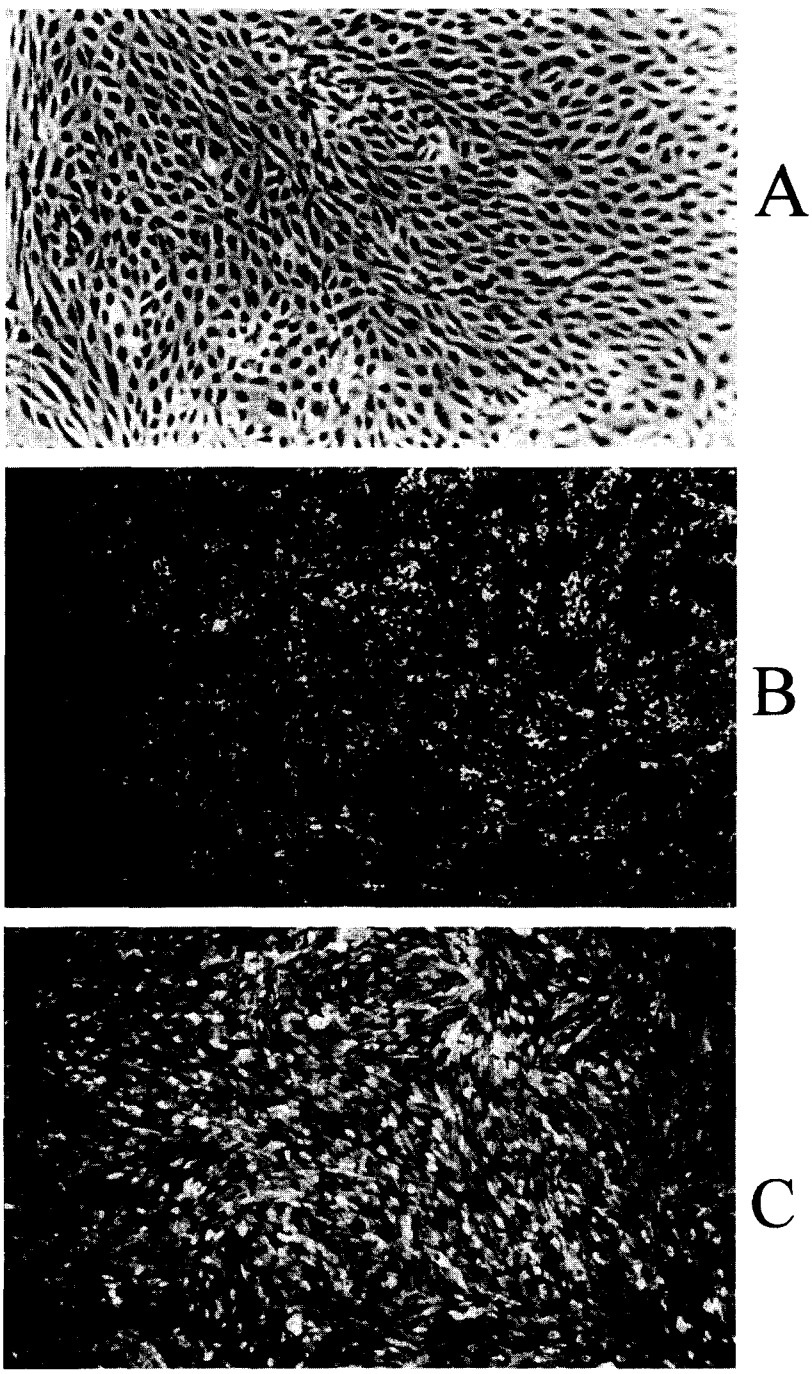

Fig. 1. Characterization of cardiac endothelial cells (CEC) in culture. CEC were grown as described in Section 2 and used in the experiments presented in Section 3. Shown are representative parallel samples identifying the cultured cells as truly endothelial. A: Light micrograph of confluent monolayer (magnification 25 ×); B: Positive staining with Dil-Ac-LDL by the method of Voyta et al. [30] (magnification 15 ×); C: positive staining with von-Willebrand factor (factor VIII) by the method of Jaffe [31] (magnification 25 ×).

thington); BSA, FCS, ADP (Boehringer Mannheim); fibronectin (Red Cross of the Netherlands); Dil-Ac-LDL (Biomedical Technologies, Stoughton USA); Anti-vWF (Dakopatts, Copenhagen); RECA1 was a kind gift from Dr. A. Duijvstijn (University of Limburg, Maastricht); phloretin, Na-heparin (Serva, Heidelberg); bovine insulin was a kind gift from Prof. A. Wollmer (Aachen), phenylarsine oxide, 2-deoxy-D-glucose (dGlc), diamide, menadione, serotonin, adrenaline, dopamine, phenylephrine, bradykinin (Sigma), histamine (RBI).

2.2. Endothelial cell isolation and culture

Isolation of cardiac endothelial cells (CEC), cultivation and characterization were carried out as described by Linssen et al. [29]. Briefly, hearts from 12-week-old male Lewis rats were perfused for 30 min with collagenase solution by the Langendorff procedure and CEC collected from the effluent by centrifugation; the cells were resuspended and washed once before they were plated on fibronectin-coated Petri dishes. After ca. 10 days cells had grown to confluence and were subcultured as follows: they were detached by trypsin treatment, resuspended in fresh culture medium, plated on fresh precoated dishes in a ratio of 1:3, and were allowed to grow to confluence. For the experiments, nearly confluent cells from the third or fourth passage were used. These cells were characterized (1) by their morphology (Fig. 1A), (2) by their ability to take up the low density lipoprotein derivative Dil-Ac-LDL [30] (Fig. 1B), (3) by immunocytochemical detection of von-Willebrand-factor [31] (Fig. 1C) and (4) of a rat endothelial cell specific antigen [32] by fluorescence microscopy (not shown).

2.3. Glucose transport assay

Three hours prior to the experiments the culture medium was removed and the cells were washed with phosphate-buffered saline (PBS; containing 0.9 mM Ca^{2+} and 0.5 mM Mg^{2+}) before Hanks' balanced salt solution (HBSS, pH 7.4) containing 4% BSA was added. Immediately before the assay, the cells were washed twice with 1 ml glucose-free HBSS. They were incubated in a final volume of 950 μl glucose-free HBSS either in the absence (control) or in presence of the agents to be tested for the indicated times (typically 15 min) at room temperature. 2-Deoxy-D-glucose (dGlc) transport was then measured by adding 50 μl of a solution containing 2 μCi 2-deoxy-D-[^{3}H]glucose and unlabelled dGlc (final concentration in the assay: 50 μM). After a period of 15 min (during which the uptake of dGlc was found to be linear; Fig. 2), transport was stopped by quickly removing the supernatant and washing the cell monolayer three times with ice-cold HBSS (containing 400 μM phloretin, a specific inhibitor of carrier-mediated glucose transport). The cells were solubilized in 0.5 ml 0.1 N NaOH/0.1% SDS and radioactivity was counted in a liquid scintillation counter (Philips). The protein content was measured in parallel samples according to the method of Lowry [33].

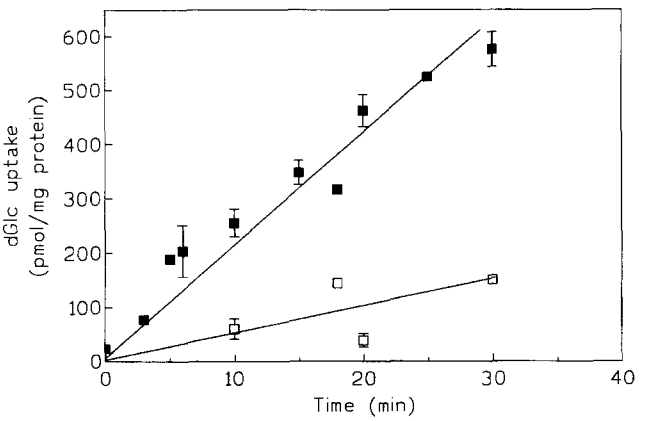

Fig. 2. Time course of dGlc uptake by CEC. Cells were preincubated for the indicated time interval in the presence (□) or absence (■) of the glucose transport inhibitor phloretin (400 μM) in glucose free HBSS, before the amount of cell-bound radioactivity was determined as described in Section 2. Values are means ± S.E.M. from 2–5 independent experiments (each experiment in triplicate).

Specific, i.e., glucose-carrier mediated uptake was determined by subtracting uptake rates measured in the presence of 170 μM phloretin from values measured in medium without phloretin. In each individual experiment, values were determined in triplicate.

2.4. Immunochemical detection of glucose transporters GLUT1 and GLUT4 by Western blot

After grown to confluence, cells were harvested with a rubber policeman in a homogenisation buffer containing: Hepes (25 mM), EDTA (4 mM), aprotinin (1 U/ml), benzamidine (25 mM), PMSF (0.2 mM), leupeptin (1 μM), pepstatin (1 μM) and sucrose (250 mM) (pH 7.4).

Cell were sonified (Branson Sonifier, Branson, Soest, FRG) and than minced 30 times in a glass/glass-homogenisator. To obtain a crude membrane fraction, the homogenate was centrifuged for 10 min at 4° C at 10 000 × g (Sorvall, SS34, Bad Nauheim, FRG). The supernatant was centrifuged again for 2 h at 4° C at 200 000 × g (Beckmann, Ti70, Fullerton, CA, USA). The resulting pellet was resuspended in a buffer containing Tris (10 mM), EDTA (0.1 mM) and SDS (2% w/v) (pH 7.5). After SDS-PAGE (10% gel), the proteins were transferred by semi-dry-blotting (Bio-Rad, Munich, FRG) to PVDF (polyvinylidine difluoride) membranes (Millipore, Eschborn, FRG). The membranes were blocked in 10% milk/PBS, 0.05% Tween 20 (pH 7.5) for 90 min and then incubated overnight with anti-GLUT1 or anti-GLUT4 antibodies (Calbiochem, Bad Soden, FRG) in 10% milk/PBS (pH 7.5) at room temperature. A crude membrane fraction from rat brain, or from rat heart were used as GLUT1- or as GLUT4-containing control, respectively. After 4 washings with PBS/0.05%

Tween 20 (pH 7.5) the membranes were incubated for 4 h at room temperature with ^{125}I-Protein A. They were then washed 6-7 times with TTBS: NaCl (137 mM), Tris (20 mM), $CaCl_2$ (2 mM), 0.05% Tween 20 (pH 7.6) and analyzed by autoradiography.

2.5. Northern blot analysis of GLUT1 and GLUT4 mRNA

The isolation of RNA was performed by the method of Chomczynski and Sacchi [54]. In brief, CEC were detached by trypsin treatment, centrifuged and dissolved in buffer containing guanidine isothiocyanate (4 M), sodium citrate (25 mM, pH 7.0), N-lauroylsarcosin 0.5%, β-mercaptoethanol (0.1 M). After extraction (twice) with phenol/chloroform at acidic pH (~4.0) and precipitation with 2-propanol, the dry RNA pellet was dissolved in sterile water and analyzed by spectrophotometry. For long-term storage at −80° C, Rnasin and dithiothreitol were added.

20 μg of each sample were subjected to formamide-formaldehyde gel electrophoresis (in 40 mM MOPS, 10 mM sodium acetate, 1 mM EDTA (pH 7.0), overnight run at 4° C at 50 mA). The RNA was transferred to a Hybond N+ nylon membrane (Amersham, Braunschweig, FRG) with capillary blot in 20 × SSPE buffer (3.6 M NaCl, 0.2 M NaH_2PO_4, [20] mM EDTA [pH 7.7]). After UV-fixation and baking (2 h, 80° C) the membranes were prehybridized in 5 × SSPE buffer supplemented with 5 × Denhardt's (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.5% SDS, 50% (v/v) formamide and 50 μl of denatured salmon sperm DNA) for at least 2 h at 42° C.

A 1.9 kb GLUT1 cDNA and a 1.5 kb GLUT4 cDNA fragment (~100 ng each; kindly provided by Prof. H.G. Joost, Aachen, FRG) were random primed labelled with α-^{32}P-dATP (25 μCi per sample; purchased from NEN, Bad Homburg, FRG; labelling kit from Boehringer Mannheim, FRG). Hybridization was carried out overnight at 42° C. The membranes were washed twice with 2 × SSPE at room temperature, once with 1 × SSPE at 50° C, dried and exposed to a hyperfilm-MP (Amersham, FRG). The same blot membranes were prehybridized again, and then hybridized with a PCR generated fragment of a glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA (which was a kind gift from Dr. Helga Rothe, Düsseldorf, FRG). The conditions of labelling, hybridization and washings were the same as for those described above.

3. Results

3.1. Carrier-mediated glucose transport in CEC

Under the experimental conditions described in Section 2, the rate of 2-deoxy-D-glucose (dGlc) uptake in CEC is linear for at least 20 min (Fig. 2). A standard incubation time of 15 min (in the presence of dGlc) was therefore chosen for all the experiments described below. In the presence of phloretin (400 μM), a specific inhibitor of glucose transport, the uptake rate of dGlc is about 20% of the value measured in the absence of this inhibitor (Fig. 2), indicating that a major part of the sugar enters the cells via a glucose carrier. In control CEC, carrier-mediated glucose uptake amounts to 347 ± 33 pmol per mg protein per min (mean from 31 independent experiments ± S.E.M.). This range is similar to values measured by others in CEC [8] or in another type of endothelial cells in culture, namely bovine aortic endothelial cells [4].

Table 1
Effect of insulin, SH-group reagents, catecholamines, and known effectors of endothelial cell function, on dGlc uptake in CEC

Addition	Agent Concentration	Relative dGlc transport rate (% of control)	Significance (vs. control)
None (control)	–	100	
Insulin (30 min)	10 nM	96.3 ± 22.8	
(60 min)		93.5 ± 6	
(90 min)		100 ± 17	
Insulin (30 min)	100 nM	104 ± 10	
Phenylarsine oxide	0.5 μM	100 ± 7	
	3 μM	69 ± 8	p = 0.08
	10 μM	44 ± 9	p = 0.01
	30 μM	16 ± 4	p < 0.001
Diamide	10 μM	114 ± 9	p = 0.18
	30 μM	104 ± 9	
	300 μM	70 ± 5	p = 0.053
	1 mM	47 ± 1	p = 0.006
Menadione	10 μM	117 ± 17	p = 0.25
	30 μM	63 ± 13	p = 0.035
	100 μM	48 ± 3	p = 0.009
Adrenaline	100 μM	91 ± 11	
Dopamine	100 μM	102 ± 2	
Phenylephrine	100 μM	103 ± 13	
Serotonin	100 μM	97 ± 1	
Bradykinin	1 μM	100 ± 6	
	10 μM	107 ± 10	
Heparin	20 U/ml	92 ± 4	
ADP	10 μM	115 ± 15	$P = 0.25$
Histamine (5 min)	100 μM	125 ± 8	$P = 0.028$
(15 min)	100 μM	132 ± 3	$P < 0.001$
(30 min)	100 μM	123 ± 1	$P < 0.001$

Cells were preincubated for 15 min in the absence (control) or in the presence of one of these agents at the indicated concentrations before dGlc uptake was measured over a period of 15 min as described in Section 2. With insulin the preincubation time ranged between 30 and 90 min, as indicated. Additional samples were treated 5 or 30 min with histamine, as indicated, the cells were then rapidly washed (once) and dGlc uptake was measured over a period of 2.5 min. Data are means ± S.E.M. from 2–4 independent experiments ($n = 3$ in each individual experiment). Statistical significance vs. control was calculated with a paired Student's t-test.

3.2. Effect of insulin, SH-group reagents, catecholamines and serotonin on glucose transport

In a first set of experiments, we tested the influence of insulin on the rate of glucose transport. As shown in Table

1, neither a preincubation of CEC with insulin (10 nM) for up to 90 min, nor a supraphysiological insulin concentration (100 nM) resulted in a stimulation of glucose transport in these cells. We also investigated the possible influence of other agents known to stimulate glucose transport in insulin-responsive cells, such as adipocytes or myocytes: SH-group reagents [11–15], catecholamines [9,16,17] and serotonin [16]. The results of the experiments in CEC are summarized in Table 1. SH-group reagents, such as phenylarsine oxide, diamide and menadione, decrease the rate of dGlc transport to about 50% of control values, at concentrations that markedly stimulate glucose transport in cardiac myocytes [15,34], adipocytes [11,13] or skeletal muscle [12,14]. Lower concentrations of these substances did not affect dGlc transport in CEC (not shown). Note that in muscle cells [12,15], as well as in fat cells [11,13], SH-reagents also exert inhibiting effects on glucose transport, but at higher concentrations than those required to depress glucose transport in CEC.

Catecholamines, such as adrenaline, dopamine or phenylephrine, and serotonin, which were shown to be potent stimulators of glucose transport in cardiac tissue [9,16,17], did not influence glucose transport in CEC (Table 1).

3.3. Effect of known stimulators of endothelial cell function

In the following series of experiments, we studied the action of metabolic and hormonal effectors on CEC glucose transport that are known to influence other endothelial cell functions: bradykinin [18–21], histamine [22–24], heparin [25,26] or ADP [27,28]. Treatment of CEC with either of these agents did not influence the dGlc uptake except for histamine (Table 1). This latter agent evoked a significant stimulation of glucose transport (Table 1). The extent of this effect, which was highly reproducible, varied from a 10% to a 50% increase of dGlc uptake, as compared to control, depending on the batch of cells.

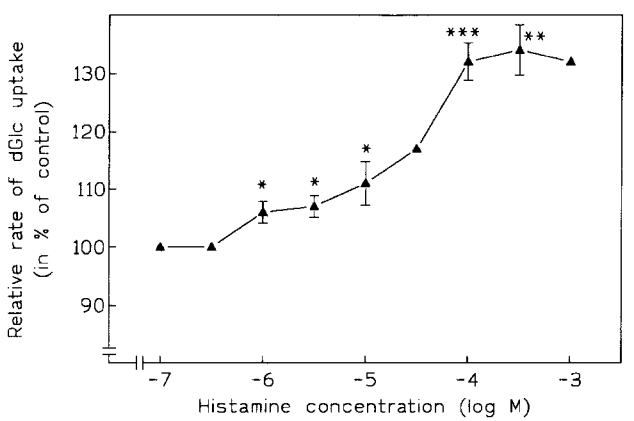

Fig. 3. Concentration dependence of the effect of histamine on dGlc uptake in CEC. The cells were exposed to the indicated histamine concentrations for 15 min in glucose free HBSS, before dGlc uptake was measured over a period of 15 min, as described in Section 2. Data are means ± S.E.M. from 2–5 independent experiments. Histamine-stimulated uptake values were compared to control (no histamine added) by one-way analysis of variance for multiple comparisons (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

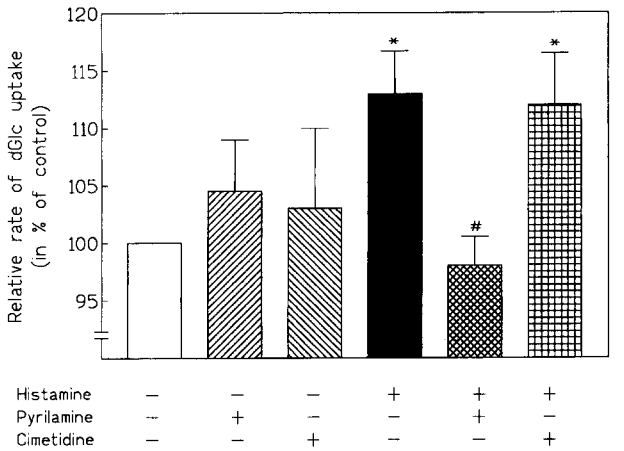

Fig. 4. Influence of H_1- and H_2-receptor antagonists on histamine stimulation of dGlc uptake in CEC. Cells were preincubated for 10 min with either 1 μM pyrilamine (a specific H_1-antagonist) or with 100 μM cimetidine (a specific H_2-antagonist) before histamine (100 μM) was added for another 5 min. dGlc uptake was then measured over a period of 10 min as described in Section 2. Values are means ± S.E.M. from 2–3 independent experiments. (*: $P < 0.05$ as compared to corresponding control by one-way analysis of variance; #: $P < 0.05$, as compared to histamine stimulated values by paired Student's t-test).

3.4. Characterization of the effect of histamine

The concentration dependence of this stimulating effect of histamine is illustrated in Fig. 3. Stimulation was half-maximal at 12 μM histamine (as calculated from linearized data from the dose–response curve shown) and reached a maximum at 100 μM (Fig. 3). The action of histamine was rapid, being maximal after 5 min (Table 1). Longer preincubation times up to 60 min with histamine did not further enhance the rate of dGlc uptake (not shown).

We next addressed the question of which receptor type might mediate the observed effect of histamine. Two of the three known histamine receptors were reported to be expressed in endothelial cells: the H_1- and the H_2-receptors [22,35]. CEC were therefore exposed to either 100 nM pyrilamine (a specific H_1-antagonist; $K_d = 0.8$ nM; [22]) or with 100 μM cimetidine (a specific H_2-antagonist; $K_d = 800$ nM; [22]) for 15 min before histamine (100 μM) was added for another 5 min. Whereas pyrilamine abolished the stimulation of glucose transport by histamine, cimetidine was without effect (Fig. 4), even at concentrations as high as 1 mM (not shown). Note that neither

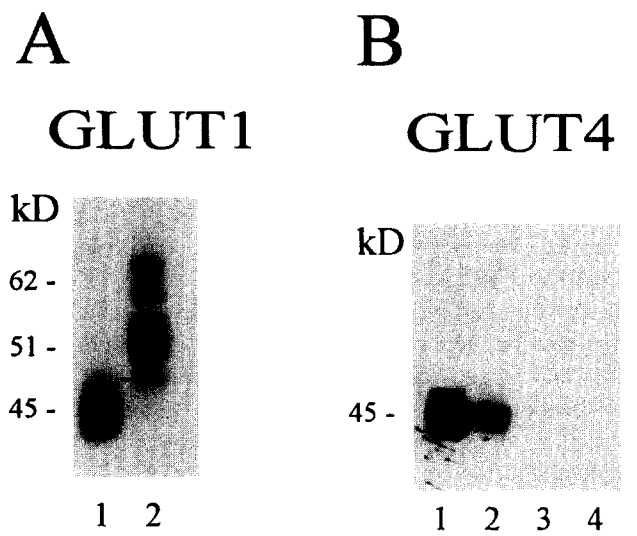

Fig. 5. Western blot analysis of CEC. A: GLUT1; lane 1: 50 μg protein of rat brain crude membrane protein, lane 2: 50 μg of CEC crude membrane protein were loaded onto the gel. B: GLUT4; lanes 1 and 2: 50 μg and 20 μg of rat heart crude membrane protein, respectively; lanes 3 and 4: 50 μg of CEC crude membrane protein from two independent cell lines. Figure shows autoradiographs of representative blots. For further details: see Section 2.

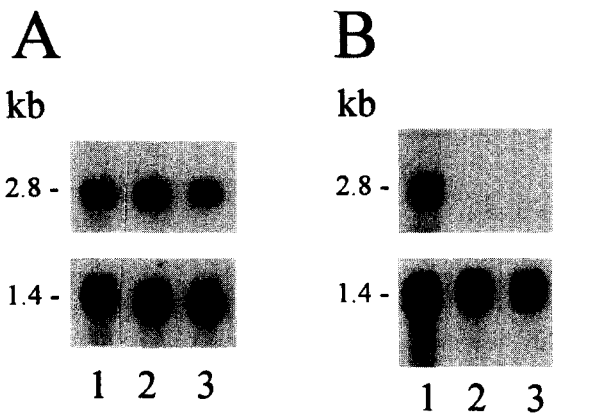

Fig. 6. Northern blot analysis of CEC. A: GLUT1; lane 1: 20 μg of rat brain total RNA (used as positive control), lanes 2 and 3: 20 μg of CEC total RNA from two independent cell cultures. B: GLUT4; lane 1: 20 μg of rat heart total RNA (positive control), lanes 2 and 3: 20 μg of CEC total RNA from two different cell cultures. Glucose transporter mRNA appear as 2.8 kb signals. The same blot membranes were re-hybridized with a GAPDH cDNA which appears as a 1.4 kb fragment in all lanes. For further details: see section 2.

antagonist affected the basal rate of glucose transport under the same conditions (Fig. 4).

3.5. Effect of combined treatment with histamine and glucose deprivation on glucose transport

In order to compare the acute action of histamine described above with the previously described long-term stimulation of endothelial cell glucose transport upon glucose deprivation [2], CEC were incubated at 37° C for 3 h in the presence or the absence of glucose before dGlc transport was measured. As shown in Table 2, glucose deprivation resulted in a 1.3-fold increase in dGlc uptake in CEC, as compared to the non-starved control. When glucose-deprived cells were exposed to a short (15 min) histamine challenge, the rate of glucose transport was a little higher than without histamine treatment, but this difference did not reach statistical significance (Table 2).

3.6. Expression of glucose transporters (GLUT1 and GLUT4) in CEC

In view of the effect of histamine on glucose transport in CEC, the question arose of the expression of the glucose transporter GLUT4. This transporter isoform is present and can be recruited in all cell types in which glucose transport is subject to acute regulation, not only by insulin [36–38], but also by other factors such as catecholamines [39] or contraction [40,41]. We therefore examined the presence of this glucose transporter, as well as of the ubiquitous carrier isoform GLUT1 in extracts of CEC by Western blot analysis. As expected, GLUT1 protein is present in these cells (Fig. 5A). Note that in CEC, GLUT1 has a somewhat higher apparent molecular weight than in brain membrane fractions used as control (Fig. 5A), which might be due to different degrees of glycosylation of the transporter protein in these tissues. In contrast, no GLUT4 protein was detectable in CEC (Fig. 5B). The same results were obtained when other anti-GLUT1 and anti-GLUT4 antibodies were used for the Western blotting (not shown). In addition,

Table 2
Effect of combined treatments with glucose deprivation and histamine on dGlc uptake in CEC

Treatment	Relative dGlc transport (in % of control)	Significance
Glucose deprivation	132 ± 8	$P = 0.028$ (vs. control) *
Histamine	113 ± 3	$P = 0.025$ (vs. control) *
Glucose deprivation + histamine	141 ± 12	$P = 0.23$ (vs. depriv. alone) * *

Cells were preincubated in the presence ('Control' or 'Histamine') or in the absence of glucose ('Glucose deprivation or Glucose deprivation + histamine') at 37° C for 3 h; they were then rapidly washed three times with glucose free HBSS before histamine (100 μM) was added for 15 min at room temperature, as indicated. dGlc uptake was then measured over a period of 15 min as described in Section 2 ($n = 3$ in each experiment). Values are means ± S.E.M. from 3 independent experiments. (*: as calculated by one-way analysis of variance; * *: as calculated by paired Student's t-test).

Northern blot analysis of CEC revealed the presence of GLUT1 mRNA in these cells (seen as a 2.8 kb fragment in Fig. 6A), whereas no signal corresponding to GLUT4 mRNA was found (Fig. 6B).

4. Discussion

The purpose of the present work was to investigate the acute regulation of glucose uptake in endothelial cells from a highly insulin-responsive organ such as the heart. To this end, isolated endothelial cells from rat hearts (CEC) were used. The major new observation of this study is the finding that histamine, an important regulator of endothelial cell function [22–24], causes an increase in glucose transport in these cells (Table 1). This effect is rapid (being maximal after 5 min of exposure to histamine) (Table 1) and persists for at least 60 min (not shown). Glucose transport stimulation by histamine does not appear to be a sign of non-specific or general stimulation of CEC since other factors known to control endothelial cell function such as bradykinin [18–21], heparin [25,26], adenosine diphosphate [27,28], and also catecholamines [42],[43] or serotonin [42–44] failed to influence the rate of glucose uptake in CEC (Table 1).

In view of the rapid onset of histamine's effect, de novo protein synthesis (e.g., of glucose transporters) is very unlikely to be required for the observed rise in glucose uptake. However, our attempt to indirectly confirm this contention was not successful. Thus, combined treatments of CEC with histamine, on the one hand, and with a protein synthesis-dependent 'long-term' stimulus of endothelial cell glucose transport, namely glucose deprivation [2], failed to reveal a statistically significant additivity of the effects of both stimuli (Table 2). A possible explanation for this absence of additivity could be a decreased sensitivity of glucose-deprived CEC towards the action of histamine.

The results of experiments performed with the selective histamine receptor antagonists pyrilamine and cimetidine (Fig. 4) clearly indicate that H_1-receptors, but not H_2-receptors mediate the action of histamine on glucose transport. In line with this notion, the EC_{50} value of transport stimulation was about 12 μM (Fig. 3), which corresponds to reported values of $\sim 10^{-5}$ M for other H_1-receptor-mediated biological responses to histamine [24,45,46], whereas H_2-receptors are documented as high-affinity binding sites ($K_d \sim 10^{-8}$ M) [22].

The other group of potential stimuli that were examined in the present work comprises substances that rapidly and markedly activate the glucose transport system of fat and muscle tissues, namely insulin [10,37,38], SH-reagents [11–15], catecholamines [9,16,17] and serotonin [16]. The lack of stimulatory effect of these agents on the uptake of glucose in CEC (Table 1), along with the absence of the 'insulin-regulatable' glucose transporter GLUT4 mRNA (Fig. 6B) and protein (Fig. 5B), seems to confirm the rule that GLUT4 expression is a prerequisite to the action of insulin and insulin-mimetic agents. As a matter of fact, in all studies in which the mechanism of an acute stimulation of glucose transport was investigated (in adipocytes, myocytes or muscle preparations), the recruitment of an intracellular GLUT4 pool to the plasma membrane was found to be involved [37–39,41,47,48]. Thus, the absence of GLUT4 in CEC would explain the inability of insulin to acutely stimulate glucose transport (Table 1), which confirms previous reports on other types of endothelial cells [2,4], and suggests that the insulin-dependent GLUT4 translocation shown by Vilaro et al. in tissue slices [5] most likely does not take place in the endothelium, but in the neighbouring fat or muscle cells.

Furthermore, the lack of GLUT4 in CEC implies that the effect of histamine occurs via a different type of mechanism than that triggered by insulin in fat and muscle or, alternatively, that it involves the translocation of another glucose transporter isoform, e.g., GLUT1 which is present in CEC (Figs. 5A,6A). Since histamine is known to induce shape changes in endothelial cells [22,24], it is conceivable that a similar process may play a role here. Such shape alterations, which are thought to be responsible for histamine's effect on the vascular permeability [22,24], were also reported to be mediated by H_1-receptors [24]. In any instance, the precise mechanism of action of histamine on glucose transport in CEC remains to be elucidated.

Finally, the effect of histamine on glucose uptake may have a biological significance in two respects. First, regarding endothelial cell metabolism, one may speculate that histamine does not only trigger the responses involved in inflammatory processes, but that it improves in parallel the supply of these cells with exogenous glucose, and thus with metabolic energy necessary to cope with acute challenges. In this context, it is worth noting that histamine was recently reported to promote glycogenolysis in astrocytoma cells via H_1-receptors with a time- and concentration-dependence reminiscent of the characteristics of the glucose transport stimulation in CEC observed in the present study [46]. Second, the endothelium constitutes a diffusion barrier for the transfer of glucose from the blood to the interstitial space, and thus to parenchymal cells. Alterations at this barrier may become physiologically significant if the parenchymal consumption of glucose is enhanced and exceeds the endothelial transfer capacity. In non-stimulated hearts, the permeability-surface area product of the capillary endothelium for D-glucose (which determines the transendothelial passage of the sugar) only exceeds that of the cardiomyocyte membrane by a factor of 1.26 (dog hearts) to 3.0 (rabbit hearts) [49]. Considering that the rate of cardiomyocyte glucose transport and utilization can be increased about 5- to 10-fold under various conditions (such as increased workload, hypoxia or insulin [15,50]), it is conceivable that transcapillary glucose transfer then becomes rate limiting. In line with this idea, the

interstitial glucose concentration of the rat myocardium was found to be decreased by interventions known to enhance cardiomyocyte glucose uptake, such as β-adrenergic stimulation [51]. Under such conditions, even a relatively modest increase in glucose transfer across the luminal endothelial cell membrane and subsequent release across the abluminal membrane may favour the transendothelial passage of the hexose. The present findings indicating a significant, albeit modest increase of glucose transport across the endothelial cell membrane supports this possibility. It is however unknown whether transendothelial cell transport plays a role of importance in cardiac tissue in vivo, or whether glucose diffusion occurs solely through endothelial clefts. In the brain, however, the endothelial barrier is tight and transcapillary exchange of glucose is exclusively mediated by endothelial cell glucose transporters [52,53], so that potential changes in glucose transport at this site could be extremely relevant. Further investigations on the glucose transport of endothelial cells originating from the brain may therefore be of considerable interest.

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References

[1] Piper, H.M., Spahr, R., Mertens, S., Krützfeldt, A. and Watanabe, H. (1990) in Cell Culture Techniques in Heart and Vessel Research (Piper, H.M., ed.), pp. 159–177, Springer, München.
[2] Takakura, Y., Kuentzel, S.L., Raub, T.J., Davies, A., Baldwin, S.A. and Borchardt, R.T. (1991) Biochim. Biophys. Acta 1070, 1–10.
[3] Loike, J.D., Cao, L., Brett, J., Ogawa, S., Silverstein, S.C. and Stern, D. (1992) Am. J. Physiol. 263, C326–C333.
[4] Pekala, P., Marlow, M., Heuvelman, D. and Connolly, D. (1990) J. Biol. Chem. 265, 18051–18054.
[5] Vilaro, S., Palacin, M., Pilch, P.F., Testar, X. and Zorzano, A. (1989) Nature 342, 798–800.
[6] Allen, L.A. and Gerritsen, M.E. (1986) Exp. Eye Res. 43, 679–686.
[7] Bar, R.S., Siddle, K., Dolash, S., Boes, M. and Dake, B. (1988) Metabolism 37, 714–720.
[8] Gerritsen, M.E. and Burke, T.M. (1985) Proc. Soc. Exp. Biol. Med. 180, 17–23.
[9] Rattigan, S., Edwards, S., Hettiarachchi, M. and Clark, M.G. (1986) Biochim. Biophys. Acta 889, 225–235.
[10] Zaninetti, D., Greco-Perotto, R. and Jeanrenaud, B. (1988) Diabetologia 31, 108–113.
[11] Olefsky, J.M. (1979) Horm. Metabol. Res. 11, 209–213.
[12] Forsayeth, J. and Gould, M.K. (1983) Biochim. Biophys. Acta 759, 184–191.
[13] Gould, G.W., Lienhard, G.E., Tanner, L.I. and Gibbs, E.M. (1989) Arch. Biochem. Biophys. 268, 264–275.
[14] Henriksen, E.J. and Holloszy, J.O. (1990) Am. J. Physiol. 258, C648–C653.
[15] Fischer, Y., Rose, H., Thomas, J., Deuticke, B. and Kammermeier, H. (1993) Biochim. Biophys. Acta 1153, 97–104.
[16] Fischer, Y., Kranz, S. and Kammermeier, H. (1992) J. Mol. Cell. Cardiol. 24 (Suppl. V), S.22.
[17] Fischer, Y., Thomas, J., Rösen, P., Kozka, I.J., Holman, G.D. and Kammermeier, H. (1994) Diabetologia 37 (Suppl. 1), 230.
[18] Furchgott, R.F. (1983) Circ. Res. 53, 557–573.
[19] Mullins, R. (1986) J. Surg. Res. 40, 540–549.
[20] Altura, B.M. and Chand, N. (1981) Br. J. Pharmacol. 74, 10–11.
[21] Cherry, P.D., Furchgott, R.F., Zawadzki, J.V. and Jothiandan, D. (1982) Proc. Natl. Acad. Sci. USA 79, 2106–2110.
[22] Hill, SJ. (1990) Pharmacol. Rev. 42, 45–83.
[23] Van de Voorde, J. and Leusen, I. (1983) Eur. J. Pharmacol. 87, 113–120.
[24] Killacky, J.J.F., Johnston, M.G. and Movat, H.Z. (1986) Am. J. Pathol. 122, 50–61.
[25] Azizkhan, R.G., Azizkhan, J.C., Zetter, B.R. and Folkman, J. (1980) J. Exp. Med. 152, 931–940.
[26] Thornton, S.C., Mueller, S.N. and Levine, E.M. (1983) Science. 222, 623–625.
[27] Furchgott, R.F. (1980) Trends in Physiol. Sci. 2, 173–176.
[28] De Mey, J.G. and Vanhoutte, P.M. (1981) J. Physiol. 316, 347–355.
[29] Linssen, M.C.J.G., Vork, M.M., Jong, Y.F., Glatz, J.F.C. and Van der Vusse, G.J. (1990) Mol. Cell. Biochem. 98, 19–25.
[30] Voyta, J.C., Via, D.P., Butterfield, C.E. and Zetter, B.R. (1984) J. Cell. Biol. 99, 2034–2040.
[31] Jaffe, E.A. (1984) in Biology of Endothelial Cells, chapter 21, pp. 209–214, Martinus Nyhoff, Boston.
[32] Duijvestijn, A.M., Goor, H.V., Klatter, F., Majoor, G.D., Van Bussel, E. and Van Breda Vriesman, P.C. (1992) Lab. Invest. 66, 459–466.
[33] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[34] Fischer, Y., Kranz, S. and Kammermeier, H. (1992) Pfluegers Arch. 420, R109/429.
[35] Takeda, T., Yamashita, Y., Shimazaki, S. and Mitsui, Y. (1992) J. Cell Sci. 101, 745–750.
[36] Slot, J.W., Geuze, H.J., Gigengack, S., James, D.E. and Lienhard, G.E. (1991) Proc. Natl. Acad. Sci. USA 88, 7815–7819.
[37] Clark, A.E., Holman, G.D. and Kozka, I.J. (1991) Biochem. J. 278, 235–241.
[38] Marette, A., Burdett, E., Douen, A., Vranic, M. and Klip, A. (1992) Diabetes 41, 1562–1569.
[39] Rattigan, S., Appleby, G.J. and Clark, M.G. (1991) Biochim. Biophys. Acta 1094, 217–223.
[40] Kolter, T., Uphues, I., Wichelhaus, A., Reinauer, H. and Eckel, J. (1992) Biochem. Biophys. Res. Com. 189, 1207–1214.
[41] Goodyear, L.J., Hirshman, M.F. and Horton, E.S. (1991) Am. J. Physiol. 261, E795–E799.
[42] Cocks, T.M. and Angus, J.A. (1983) Nature 305, 627–629.
[43] Gerritsen, M.E. (1987) Fed. Proc. 46, 47–53.
[44] Cohen, R.A., Shepherd, J.T. and Vanhoutte, P.M. (1983) Am. J. Physiol. 245, H1077–H1080.
[45] Donaldson, J. and Hill, S.J. (1987) Br. J. Pharmacol. 90, 263–271.
[46] Medrano, S., Gruenstein, E. and Dimlich, R.V.W. (1992) Brain Res. 592, 202–207.
[47] Vogt, B., Mushack, J., Seffer, E. and Haring, H.U. (1990) Biochem. Biophys. Res. Commun. 168, 1089–1094.
[48] Fischer, Y., Thomas, J., Rösen, P. and Kammermeier, H. (1995) Endocrinology, in press.

[49] Kuikka, J., Levin, M. and Bassingthwaighte, J.B. (1986) Am. J. Physiol. 250, H29–H42.
[50] Zaninetti, D., Greco-Perotto, R. and Jeanrenaud, B. (1988) Diabetologia 31, 108–113.
[51] Kammermeier, H. and Wendtland, B. (1987) J. Mol. Cell. Cardiol. 19, 167–175.
[52] Crone, C. (1965) J. Physiol. 181, 103–113.
[53] Maher, F., Vannucci, S.J. and Simpson, I.A. (1994) FASEB J. 8, 1003–1011.
[54] Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.