Mechanical stress and glucose concentration modulate glucose transport in cultured rat podocytes

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Abstract

Background. Recent studies show that mechanical stress modifies both morphology and protein expression in podocytes. Ambient glucose is another factor modulating protein synthesis in these cells. In diabetes, podocytes experience elevated glucose concentrations as well as mechanical strain generated by high intracapillary pressures. Both these factors are responsible for podocyte injury, leading to impairment of kidney glomerular function. In the present study, we examined the effects of glucose concentration and mechanical stress on glucose uptake in podocytes.

Methods. Following a 24 h pre-incubation in low (2.5 mM, LG), normal (5.6 mM, NG) or high (30 mM, HG) glucose media, cultured rat podocytes were exposed to 4 h mechanical stress. We used the labelled glucose analogue, [3H]2-deoxy-D-glucose, to measure glucose uptake. The distribution of facilitative glucose transporters GLUT2 and GLUT4 was assessed by flow cytometry.

Results. In the control (static) cells, glucose uptake was similar in the three glucose groups. In mechanically stressed podocytes, glucose uptake increased 2-fold in the LG and NG groups but increased 3-fold in the HG group. In the NG cells, mechanical load increased the membrane expression of GLUT2 and reduced the membrane-bound GLUT4. In stretched HG cells, the membrane expression of both GLUT2 and GLUT4 was decreased. High glucose decreased the plasma membrane GLUT2 content in the stretched cells, whereas both static and stretched podocytes showed an elevation in GLUT4.

Conclusion. Mechanical stress potentiated glucose uptake in podocytes and this effect was enhanced by high ambient glucose. The decreased expression of GLUT2 and GLUT4 on the surface of stretched cells suggests that the activity of other glucose transporters may be regulated by mechanical stress in podocytes.

Keywords: cultured podocytes; GLUT2; GLUT4; high glucose; mechanical stress

Introduction

Podocytes are highly specialized cells that make up part of the glomerular filtration barrier. Their foot processes, which are fixed to the glomerular capillaries on the outer aspect of the glomerular basement membrane (GBM), counterbalance the intracapillary pressure [1]. Moreover, podocytes also support the capillary tuft by synthesizing components of the GBM [1]. Susceptibility of podocytes to mechanical forces has been shown in vitro and in vivo. For example, biaxial mechanical stress applied to cultured podocytes induced changes in cell morphology, caused a reduction in proliferation, and modulated the expression of several genes [2]. During in vivo studies, glomerular hypertension and hyperfiltration were associated with degenerative changes in podocytes, leading to progressive damage of glomeruli [3].

In diabetic nephropathy, podocytes are exposed not only to increased intracapillary pressure but also to elevated glucose concentrations. To date, there is no evidence for direct toxic effects of glucose on podocytes; however, hyperglycaemia modifies the production of several proteins in these cells [4,5]. In humans, diabetic glomerular lesions were accompanied by podocyte loss [6].

Mechanical stress-dependent stimulation of glucose transport in skeletal muscle has been well documented [7]. In addition, exposure to high glucose...
causes autoregulation of glucose uptake in different cells types, and this can be up- [8] or downregulated depending on the duration of hyperglycaemia and on cell type [9].

However, it is not yet clear whether there is a direct pathway between extracellular glucose and intracellular events in podocytes. The first step in such a pathway is the mechanism(s) mediating glucose entry into the cell. We recently have characterized glucose transport as well as the presence of several trans- port systems in cultured rat podocytes [10]. In the present study, we examined the effects of mechanical stress and glucose concentration on glucose uptake in podocytes.

Subjects and methods
Isolation and culture of podocytes from kidney glomeruli
For these experiments, we used female Wistar rats weighing 120–140 g that received a standard rat chow. The podocytes were isolated from rat glomeruli as previously described [11]. Briefly, the glomeruli were grown in culture flasks for 5–7 days. The outgrowing epithelial cells were trypsinized, passed through a sieve having a 33 μm pore size, and resuspended in RPMI 1640 medium (Sigma Chemicals Co, St Louis, MO) supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Paisley, UK), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma). The cells were seeded in 6-well plates having a flexible bottom (Bioflex, Flexcell International Hillsborough, NC) and cultivated at 37°C for 12–20 days. The phenotype of podocytes was checked routinely using podocyte-specific antibodies to Wilm’s tumour-1 protein (WT-1; Biotrend Koeln, Germany) and synaptopodin (Progen, Heidelberg, Germany). Cell viability was monitored by lactate dehydrogenase leakage using a kinetic method [12] and was not less than 85%.

Mechanical stress experiments
At 24 h prior to stretching, the standard culture medium was replaced by glucose-free RPMI 1640 (Sigma) supplemented with antibiotics as well as 10% FBS, and low (LG, 2.5 mM), normal (NG, 5.6 mM) or high (HG, 30 mM) D-glucose. To obtain an equal osmolality level in all media, the LG and NG media were supplemented with mannitol. Fresh media containing 0.2% bovine serum albumin (Sigma) instead of FBS were added at 60 min prior to mechanical stressing, and the plates were mounted on a manifold connected to the stretch apparatus (StretchCo, Edingen, Germany). Variations in air pressure caused cyclic vertical oscillations of the flexible plate bottom that had a frequency of 0.5 Hz and maximum linear strain of 5%. This frequency was chosen because autoregulation of renal blood flow is capable of damping pressure fluctuations below 0.1 Hz, whereas faster pressure fluctuations are fully transmitted to glomerular capillaries. Certain cells were subjected to mechanical strain for a period of up to 4 h, whereas others, serving as controls, were grown in wells with flexible membranes but were not subjected to mechanical stress. The osmolality of the media, checked before and after the stretch experiments, was maintained at 290 ± 10 mOsm.

Glucose uptake
Glucose uptake was measured in the LG, NG and HG media for a period of 1–4 h, using 1 μCi/well of [3H]2-deoxyglucose (3H-2DG) that had a specific activity of 29.8 Ci/ mmol (NEN-DuPont, Boston, MA). Incubation was terminated by rapidly removing the supernatant, washing the podocytes with ice-cold phosphate-buffered saline (PBS) free of Ca2+ and Mg2+, and lysing the cells in 0.05 M NaOH with shaking for 60 min at room temperature. Incorporated radioactivity was determined by liquid scintillation counting (Wallac 1409). A 0.1 ml aliquot was used to determine the protein concentration using a modified Bradford method [13].

Flow cytometry
Following the 4 h mechanical stress in period standard RPMI 1640 medium, the cells were washed with PBS, trypsinized, suspended in PBS and centrifuged twice for 7 min each at 400 g at 4°C. Finally, the pellet was resuspended in cold PBS and aliquots of 1×105 cells/tube were stained for 30 min at 4°C with 1:100 diluted rabbit antibodies to GLUT2 and GLUT4 (Santa Cruz Biotechnology, Santa Cruz, CA). The surface-bound antibodies were visualized using appropriate Cy-2-conjugated donkey anti-rabbit IgG (Rockland, Gilbertsville, USA).

For measurement of intracellular expression of GLUT proteins, the surface-stained cells were fixed-permeabilized in methanol for 20 min at −20°C, washed once in cold PBS, and incubated with the same primary anti-GLUT antibodies as used for the surface staining. In order to distinguish between surface and intracellular expression of glucose transporters, we used a different secondary antibody that was conjugated to orange fluorescent phycoerythrin (Accurate Chemical & Scientific Corp., USA). The specificity of staining was confirmed by using an irrelevant rabbit antiserum in place of the anti-transporter antibodies. We found that there was no non-specific binding of irrelevant rabbit immunoglobulins to the surface or to the intracellular components of rat podocytes. Furthermore, fluorochrome-conjugated secondary antibodies displayed negligible unspecific staining of viable or fixed-permeabilized podocytes.

Surface- and cytoplasm-stained cells were washed twice with cold PBS, resuspended in 1 ml of PBS and analysed by flow cytometry (Galaxy Flow Cytometry System, Dako, Denmark). A total of 15 000 cells were collected from each tube and analysis was performed off-line using WinMDI 2.9 (Joseph Trotter, The Scripps Institute, La Jolla, CA). An arbitrary selection of GLUT-positive cells was performed by baseless measurements on single-cell fluorescence intensities. For each selected population, a mean fluorescence index (MFI) was determined.

Statistical analysis
Results are expressed as means± SE from not less than three experiments. Statistical analysis was performed using Mann–Whitney and Student t-tests.
Results

Effect of glucose and mechanical stress on glucose uptake by rat podocytes

In the non-stretched podocytes, uptake of 3H-2DG was steady during the duration of the experiment and was not affected by glucose concentration changes in the medium (Figure 1). The stretched podocytes in LG and NG media had glucose accumulation that was similar to controls, but only during the first 2 h stretching. After 4 h, the stretched cells showed a rapid increase in glucose accumulation. In contrast, podocytes stretched in the HG medium showed higher glucose uptake even after the first hour of stretching. After 4 h, glucose uptake in these cells was 3.5-fold higher than in their respective controls (0.67 ± 0.13 vs 0.20 ± 0.03 μmol/mg protein, n = 4, P < 0.01).

The present results indicate that under these experimental conditions, the greatest response to mechanical stress in podocytes occurred between the second and fourth hour of stretching. In the non-stretched cells, there were no significant differences in glucose uptake between the LG, NG and HG groups. In contrast, the stretched podocytes showed an ~2-fold (LG and NG) and 3-fold (HG) greater glucose uptakes than the respective control cells. Moreover, glucose uptake in the stretched HG cells significantly exceeded the uptake in the stretched LG and NG cells (n = 5, P < 0.05).

Effect of mechanical stress on distribution of GLUT2 and GLUT4 in podocytes

Since glucose uptake was similar in podocytes incubated in the LG and NG conditions, the distribution of GLUT2 and GLUT4 was checked in cells from the NG and HG groups. As shown in Figure 2, mechanical stressing for 4 h significantly increased (by 29.2 ± 8%) the membrane expression of GLUT2 in NG cells but...
decreased (by 20.4±5%, \( P < 0.05 \) vs static cells) the membrane content of GLUT2 in the HG group. The expression of GLUT4 on the cell surface was reduced by mechanical stress in both the NG and HG groups. Tables 1 and 2 summarize the effects of mechanical stress on the subcellular distributions of GLUT2 and GLUT4, shown as percentages of GLUT-positive cells and as MFIs of selected cell populations (see Materials and methods). The MFI value reflects the mean GLUT density on the surface of or inside a single podocyte.

### Discussion

In recent previous work [10], we showed that under basal conditions (non-stretched cells cultured in the standard RPMI 1640 medium), the majority of facilitated glucose transport protein GLUT2 and GLUT4 expression was localized intracellularly. In the current study, we examined whether conditions mimicking the diabetic state modulate glucose uptake into podocytes and whether the GLUT2 and GLUT4 transporters are involved in this process.

In diabetes, two major pathogenic factors impacting on kidney glomeruli are elevated glucose concentrations and capillary hypertension. In the latter, mechanical forces are transferred to all glomerular cells including podocytes. Therefore, we compared the functional activity of glucose transporters in podocytes that were incubated in low (LG), normal (NG) and high glucose (HG) media and subjected to mechanical stress. Stretching induced an increase in glucose uptake in all groups, and the HG environment augmented this effect (Figure 1). The rate of facilitated glucose transport into the cells is related to the number of GLUT transporters trafficking to the cell surface. In our experiments, induced mechanical stress was accompanied by an increase in the membrane GLUT2 content in NG cells (Figure 2). In contrast, stretched...
podocytes exposed to HG showed a significant drop in the surface expression of GLUT2, and the surface GLUT4 content decreased in both the NG and HG groups. These findings suggest that GLUT2 may be involved in stretch-dependent increases in glucose uptake, whereas the augmenting effect of high glucose on glucose transport may be mediated by transporters other than GLUT2 and GLUT4. In addition, even though the HG group had a markedly elevated surface GLUT4 expression (Figure 3), glucose by itself did not significantly change glucose transport (Figure 1). Hence, it is likely that mechanical stress could trigger a mechanism resulting in intensification of glucose uptake in the HG medium.

In our previous study [10], we demonstrated that podocytes express mRNA not only for GLUT2 and GLUT4 but also for GLUT1, GLUT3 and SGLT1. It is therefore possible that activation of glucose uptake in stretched HG cells was due to activation of this differentiated glucose transport system. Such activation may involve not only increased GLUT trafficking and/or intrinsic activity, but also synthesis of transporter proteins. It has been shown previously that mechanical stress modulates the expression of numerous genes in podocytes [2], and this includes an upregulation of transforming growth factor-β (TGF-β) [14]. Because mechanical stretch in mesangial cells upregulated glucose transport and GLUT1 expression through an effect mediated by TGF-β [15], a similar GLUT1 upregulation may occur in podocytes. An increased podocyte capacity for glucose transport and an enhanced ability to meet increased energy demands may also be due to upregulation of GLUT3, a high affinity and high activity transporter [16]. In addition, both high glucose and mechanical stress act through similar mechanisms and many of these actions produce activation of protein kinase C to result in altered gene transcription [17]. Increased glucose transport in stretched HG podocytes may therefore be the result of molecular changes induced by both these stimuli acting in concert. However, the decreased surface expression of low affinity and high capacity GLUT2 in HG cells may represent a protective mechanism against excessive glucose uptake during hyperglycaemic conditions.

In glomerular mesangial cells [18] as well as in podocytes [19], previous studies have described associations between hyperglycaemia and mechanical strain during the pathogenesis of diabetic nephropathy. It has also been shown that development of diabetic nephropathy is dependent predominantly on haemodynamic factors [20]. The present study, showing effects of high glucose and mechanical stress on glucose uptake, appear to be in accord with these findings. An increase in intracellular glucose concentration in stretched podocytes may trigger the synthesis of typical hyperglycaemic factors leading to cell and glomerular impairment. These findings support the concept that glomerular hypertension in diabetes may be crucial for altering podocyte function and for causing underlying glomerular injury.

In summary, we demonstrated that mechanical stress stimulates glucose uptake in podocytes and that this effect is enhanced by high ambient glucose. Furthermore, both stretching and high glucose modified the subcellular distribution of GLUT2 and GLUT4, but each in a different manner. Further studies will be required to elucidate the mechanisms of glucose transporter modulation in podocytes subjected to mechanical strain and hyperglycaemia.

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