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Activity and expression of the Na^+/H^+ exchanger in human endothelial cells cultured in high glucose

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Summary Establishing whether high ambient glucose affects the plasma membrane Na^+/H^+ exchanger is relevant to understanding the adverse effects of high glucose on cell replication and the mechanisms of the increased exchanger activity encountered in diabetic patients with nephropathy. In 8 primary and 15 first-passage isolates of human endothelial cells cultured in 30 mmol/l glucose for 8.7 ± 2.3 and 15.8 ± 2.3 days, respectively, we determined Na^+/H^+ exchanger activity and mRNA levels. Activity was determined by measuring $^{22}Na^+$ influx in the presence or absence of dimethylamiloride (DMA) after intracellular acidification. We also measured fibronectin mRNA because fibronectin provides signals for cell replication through the Na^+/H^+ antiporter. Control cells grown in 5 mmol/l glucose showed at morphologic confluency a total Na^+ influx (in nmol · mg protein^{-1} · min^{-1}) of 10.1 ± 3.2 in primary and 11.7 ± 2.2 in first subculture, which was reduced to 5.3 ± 0.3 in the presence of DMA. Paired cultures exposed to 30 mmol/l glucose and exhibiting pHi and cell densities identical to controls showed in both primary and first subculture a reduction in total Na^+ influx ($\Delta = -0.98 \pm 0.93$ nmol · mg protein^{-1} · min^{-1} $p < 0.005$) whereas DMA-resistant Na^+ influx was identical to that of control. Neither chronic hypertonicity nor acute exposure to high glucose mimicked the effects of chronic high glucose. The level of the Na^+/H^+ exchanger isoform 1 (NHE-1) mRNA was unchanged by high glucose whereas fibronectin mRNA levels were increased 1.5-fold. These studies indicate that in endothelial cells exposed to elevated ambient glucose the regulation of the Na^+/H^+ exchanger is altered at the post-transcriptional level; decreased activity of the antiporter is concomitant with fibronectin overexpression and may contribute to the decreased replication caused by high glucose. [Diabetologia (1995) 38: 785–791]

Key words Na^+/H^+ exchanger, endothelial cells, cell replication, fibronectin, diabetic nephropathy.

The Na^+/H^+ exchanger is a ubiquitous transmembrane protein that under physiological conditions catalyses the entry of extracellular Na^+ in exchange for intracellular H^+ equivalents. The system regulates intracellular pH, cellular volume, transepithelial transport of acid-base equivalents, and plays a role in modulating cellular responses to mitogenic stimuli [1].

Elevated glucose concentrations have been shown to hamper in vitro the proliferation of several cell types [2] and to delay the transit of human endothelial cells through G1 [3], the phase of the cell cycle during which growth factors activate events in preparation for DNA synthesis [4]. Insofar as the effects of high glucose on endothelial cell replication are not specific for any given growth factor [3], they may be exerted at the level of molecules transducing the mitogenic signals. Among these molecules the Na^+/H^+ exchanger is prominent because, although the precise significance of its activation by growth factors in

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Abbreviations: DMA, dimethylamiloride, pHi, intracellular pH, BSA, bovine serum albumin; PCR, polymerase chain reaction; PKC, protein kinase C; NHE-1, Na^+/H^+ exchanger isoform 1.

mammalian cells exposed to physiological media has not been clarified [1], the consistency of its activation bodes for at least permissive or facilitatory effects early in the proliferative process. The exchanger is responsive not only to growth factors, but also to adhesive interactions between cells and the extracellular matrix. Namely, clustering of the fibronectin-specific integrin receptor $\alpha 5\beta 1$ by insoluble fibronectin leads to exchanger activation [5]. Because endothelial cells exposed to high glucose upregulate fibronectin synthesis [6, 7], and also overexpress $\alpha 5\beta 1$ integrin [8], it was of interest to determine whether this cellular phenotype – characterized by decreased proliferation [3] and firmer adhesion to matrix [8] – exhibits modified activity of the Na^+/H^+ exchanger.

An additional reason for inquiring whether high ambient glucose can alter the regulation of the Na^+/H^+ exchanger is the proposed connection between abnormal exchanger activity and the development of diabetic renal disease. Recent studies report increased activity of the Na^+/H^+ exchanger in leucocytes [9], erythrocytes [10], and skin fibroblasts [11] of patients with diabetic nephropathy. If the observations made in cultured fibroblasts [11] – several generations removed from the abnormal diabetic milieu – strongly support the contention that abnormal exchanger function reflects individual genetic characteristics of the antiporter or of its regulation, other findings are compatible with modulation of its activity by the diabetic environment. The raised Na^+/H^+ exchanger activity of leucocytes from diabetic patients with albuminuria appears to be mediated by increased activity of protein kinase C [9], which may be consequent to hyperglycaemia [12]. Moreover, acute changes in medium osmolarity have been shown to activate the Na^+/H^+ exchanger to drive a volume regulatory response in many cell types [1].

In this study we report on the effects of glucose concentrations mimicking diabetic hyperglycaemia on the Na^+/H^+ exchanger of human endothelial cells and on the activity of the exchanger in isolates overexpressing fibronectin in response to high glucose.

Materials and methods

Cell culture. To study the effect of exposure to high glucose for different lengths of time both primary and first-passage cultures of human endothelial cells were used. Umbilical cords were obtained from normal pregnancies, the donors remained anonymous. Each culture was derived from an individual umbilical vein; cells were plated in 35- or 60-mm tissue culture dishes for functional studies of the Na^+/H^+ exchanger or RNA extraction, respectively. Seeding density for cells destined to chronic high glucose (30 mmol/l) treatment was 30% greater than for control cells (5 mmol/l glucose). As verified in previous studies [6, 13] the differential plating density offers the means with which to study all the cells from the same culture at similar final density despite the replicative delay induced by high glucose [3]. First-passage cells cultured in high glucose were plated from dishes maintained in high glucose throughout primary culture. In selected experiments, cultures companion to those treated with high and normal glucose were treated with ribitol (25 mmol/l) to control for the chronic effect of hypertonicity, and other cultures were exposed to high glucose for only 24 h before harvest to investigate the acute effects of high glucose. Cells were cultured as previously described [13] in medium 199 (Gibco, Grand Island, N.Y., USA) supplemented with 14% heat-inactivated pooled human sera, 20 μg/ml endothelial growth supplement (Sigma, St. Louis, MO., USA), 90 μg/ml heparin (Gibco), 2 mmol/l glutamine, and 17.5 mmol/l HEPES buffer. Cultures were studied upon achievement of morphologic confluency.

Measurement of Na^+/H^+ exchange. The activity was determined by measuring unidirectional Na^+ influx stimulated by cellular acidification in the presence or absence of dimethylamiloride (DMA). Na^+ influx was measured using ^{22}Na as previously described [14]. Briefly, confluent monolayers were washed three times with Na^+-free buffer containing (in mmol/l) 130 choline chloride, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 20 Tris/HEPES pH 7.0 at 37 °C, osmolarity 300 ± 10 mOsm), and equilibrated for 25 min in the Na^+-free buffer containing 10 mmol/l glucose and 1 mg/ml bovine serum albumin (BSA). This procedure reduces intracellular Na^+ and lowers basal pHi from approximately 7.2 to 6.9 (see Results) as previously observed in both human umbilical vein endothelial cells [15] and rat aortic smooth muscle cells [14], and augments Na^+ influx twofold [14]. The monolayers were incubated for an additional 5 min with 1 mmol/l ouabain and 0.1 mmol/l bumetanide to block the Na^+/K^+ pump and the $Na^+/K^+/Cl^-$ cotransporter, respectively, both very active in endothelial cells [16]. To initiate the transport measurements, the equilibration medium was replaced by influx medium containing 2 μ Ci/ml ^{22}Na (Amersham, Arlington Heights, Ill., USA), and (in mmol/l) 1 ouabain, 0.1 bumetanide, 100 NaCl, 35 choline chloride, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 20 HEPES pH 7.4; with or without 20 μmol/l DMA, supplied by Dr. E. Cragoe of Merck Sharp & Dohme, Rahway, N.J., USA. The ^{22}Na influx – which is linear for up to 10 min in endothelial cells [15] – was terminated after 5 min by aspiration of the medium followed by four washes with an ice-cold solution containing 100 mmol/l $MgCl_2$ and 20 mmol/l 3-N-morpholino-propane sulfonic acid (MOPS, Sigma), pH 7.4 at 4 °C. Cells were extracted with 0.2% sodium dodecyl sulfate; radioactivity was counted in a gamma counter, and total protein content was determined according to the Lowry procedure [17].

Measurement of intracellular pH. Intracellular pH (pHi) of first-passage cells cultured in normal or high glucose for 14.3 ± 2.5 days was measured both at rest and at the end of the acidification protocol used to activate the Na^+/H^+ exchanger. Undisturbed monolayers were loaded with the pH-sensitive fluorescent dye 2′, 7′ – bis – (carboxyethyl) 5, (6′) carboxyfluorescein pentaacetoxymethyl ester (BCECF-AM, Calbiochem, San Diego, Calif., USA) [18, 19] at a concentration of 5 μmol/l in complete medium 199 for 30 min at 37 °C. Measurements were performed both in cells attached to the plastic coverslips (cut from Petri dishes) on which they had grown to confluency, and in cell suspensions obtained by trypsinization of confluent monolayers. Coverslips or cell suspensions were transferred to a thermostatted (37 °C) quartz cuvette in a Perkin-Elmer LS 50 Luminescence Spectrometer (Perkin-Elmer Cetus, Norwalk, Conn., USA) and either kept in HEPES-buffered saline consisting of (in mmol/l) 140 NaCl, 5 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$, 5 glucose, 15 HEPES, 1 g/l BSA, pH 7.4 at 37 °C, for measurement of basal pHi, or incubated in Na^+-

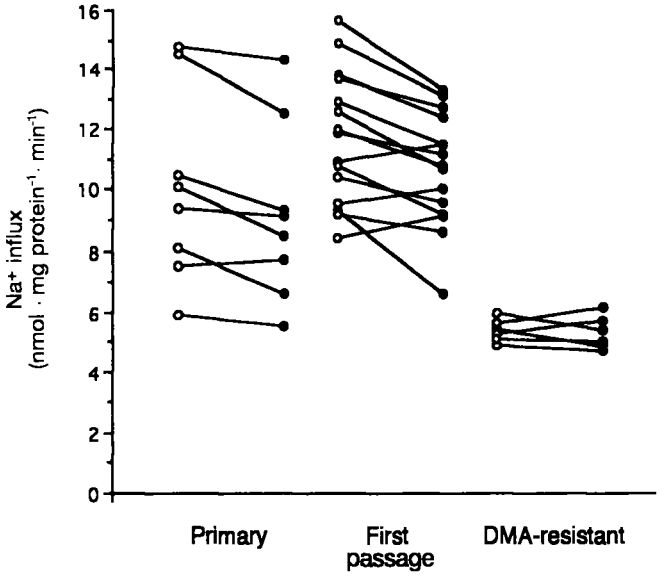

Fig. 1. Effects of high glucose on total and DMA-resistant Na^+ influx in 8 primary and 15 first-passage isolates of human endothelial cells. Each symbol represents an isolate originated from a single umbilical cord and the data from the same isolate cultured in 5 (○) or 30 (●) mmol/l glucose are connected by a line. Duplicate dishes from each treatment were tested in all experiments. Exposure to 30 mmol/l glucose was for 8.7 ± 2.3 days for primary and 15.8 ± 2.3 days for first-passage cultures. Of the cultures tested with DMA, two were primary and four first-passage

free buffer for measurements in acid-loaded cells. The dye was alternately excited at 492 and 438 nm, and fluorescence emission was measured at 530 nm for 10 min for basal pHi measurement or throughout the 30 min incubation in Na^+-free buffer used in the acidification protocol to activate the Na^+/H^+ exchanger. The 492 : 438 ratios were converted to pHi values with a calibration curve generated by exposing cells to KCl buffer consisting of (in mmol/l) 140 KCl, 1.8 $CaCl_2$, 0.8 $MgSO_4$, 5 glucose, 15 HEPES, pH 7.6–6.8) in the presence of 5 μmol/l nigericin (Sigma) and 5 μmol/l monensin (Calbiochem). The pHi value of each experimental sample was determined from the calibration curve generated with the same sample. Autofluorescence was minimal and identical in cells cultured in normal and high glucose.

Measurement of mRNA levels. mRNA studies were performed to investigate (i) the effects of high glucose on Na^+/H^+ exchanger expression and (ii) to correlate glucose-induced changes in Na^+/H^+ exchanger activity or expression with changes in the expression of fibronectin. For the study of Na^+/H^+ exchanger mRNA levels, experiments performed on RNA extracted [20] from monolayers companion to those tested for exchanger activity showed that exchanger mRNA could not be visualized in Northern analysis of total RNA. Hence RNA extracted from 21 additional first-passage isolates was used to generate RNA pools from which poly (A^+) RNA could be isolated. The RNA aliquots contributed by the high-glucose cells and their paired controls to the respective pools were identical. Poly (A^+) RNA was isolated by hybridization to a biotinylated oligo (dT) primer (Poly ATract mRNA Isolation Systems, Promega Corporation, Madison, Wis., USA). For the correlative studies, the fibronectin transcript was measured in total RNA extracted from first-passage cultures companion to those tested for Na^+/H^+ exchanger activity and in the same poly (A^+) RNA tested for Na^+/H^+ mRNA levels.

Total RNA and poly (A^+) RNA were electrophoresed on 1 % agarose gel containing 2.2 mol/l formaldehyde, and Northern blots [6] were hybridized to ^{32}P-labelled cDNAs (Multiprime DNA labeling systems, Amersham) for human fibronectin [21], γ-actin [22], and Na^+/H^+ exchanger. The 613-nucleotide Na^+/H^+ exchanger cDNA representing residues 1889–2501 of the full-length cDNA encoding NHE-1 [23] was prepared by reverse transcription of human lymphocyte RNA and amplification by polymerase chain reaction (PCR) using the following primers: 5′–AGATCCGCAAAATCCTGAGGA-3′ (upstream) and 5′ – CTGCTCTGGTGGAAGAGTCTG-3′ (downstream). PCR was performed in a Perkin-Elmer Cetus DNA thermal cycler at the following cycle conditions: denaturation 1 min at 95 °C, annealing 1 min at 60 °C, and extension 1 min at 72 °C for 30 cycles. The specificity of the PCR product was verified by separate digestions with several restriction endonucleases (Bam HI, Ban I, Hae II, Hinf I, and Pvu II, all from New England Biolabs, Beverly, Mass., USA) and electrophoresis in 2 % agarose. In all instances the sizes of the resulting restriction fragments were as predicted by the restriction map of the sequence of interest in the human Na^+/H^+ exchanger cDNA.

Hybridization and washing of Northern blots have been described [6]; exposure to Kodak XAR-5 film at –80 °C was for 12–24 h. Densitometric analysis of autoradiograms was performed at non-saturating exposures with a laser scanning densitometer and the readings of experimental samples were expressed as a percentage of readings in paired control samples after correction for loading inequalities with the actin signal. The latter was used as an internal control because the actin mRNA levels are not changed by high glucose [7, 13].

Statistical analysis

The data were summarized with the mean ± 1 SD. Statistical analysis of the glucose-induced changes in Na^+ influx and mRNA levels was performed with the two-tailed Student's *t*-test (paired analysis) and the Wilcoxon signed-rank test, respectively. Comparison of Na^+ influx in cultures subjected to several different treatments was by analysis of variance (ANOVA) followed by a multiple comparison procedure (Fisher).

Results

Na^+ influx was measured in a total of 23 cultures. The eight primary cultures had been exposed to high glucose for 8.7 ± 2.3 days; total protein levels at harvest were 280 ± 50 μg/dish vs 286 ± 60 μg/dish in the control cultures, indicating identical cell density insofar as high glucose does not change total protein synthesis in these cultures [3]. The identical final cell density was achieved by plating cells destined to high glucose treatment at greater density to compensate for their prolonged cell-cycle traversal [3]. Total Na^+ influx (Fig. 1) in the primary cultures exposed to high glucose was 9.2 ± 2.8 vs 10.1 ± 3.2 nmol · mg protein^{-1} · min^{-1} in paired control cultures ($p < 0.01$). Similar results were obtained in 15 first-passage cultures exposed to high glucose for 15.8 ± 2.3 days. In

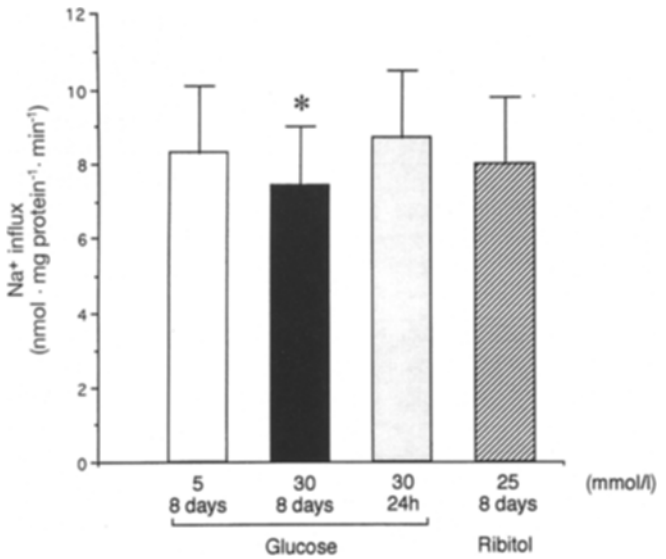

Fig. 2. Effects of acute high glucose and chronic hypertonicity (ribitol) on total Na^+ influx in human endothelial cells. Each bar represents the mean ± SD of the observations in four isolates; each determination was made in duplicate. $*p < 0.05$ vs 5 mmol/l glucose

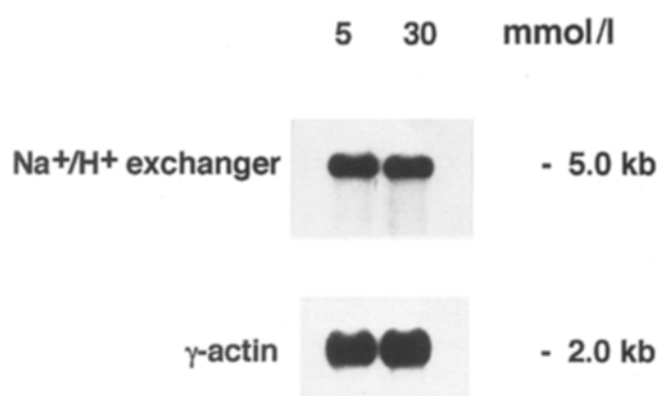

Fig. 3. Northern analysis of human endothelial cell Na^+/H^+ exchanger and γ-actin. Poly(A^+)RNA was isolated from five first-passage cultures exposed to 5 or 30 mmol/l glucose for 14 ± 2 days, and electrophoresed (9 μg/lane) and hybridized as described in Methods

these cultures total protein levels at harvest were 183 ± 71 vs 187 ± 54 μg per dish in control cultures; and total Na^+ influx was 10.8 ± 1.9 vs 11.7 ± 2.2 nmol · mg protein^{-1} · min^{-1} in control cultures ($p < 0.01$). The glucose-induced decrease in total Na^+ influx was evident in 7 of the 8 primary and 12 of the 15 first-passage cultures (Fig.1); the decrease in the two groups combined was –0.98 ± 0.93 nmol · mg protein^{-1} · min^{-1} ($p < 0.005$).

To determine which fraction of the Na^+ influx was driven by the Na^+/H^+ antiporter, we studied the effect of DMA, an amiloride analogue that potently inhibits the Na^+/H^+ exchanger [24]. The fraction of Na^+ influx measurable in the presence of 20 μmol/l DMA was identical in high-glucose and control cultures (5.3 ± 0.5 and 5.3 ± 0.3 nmol · mg protein^{-1} · min^{-1} respectively) (Fig. 1), whereas in the same six cultures the DMA-sensitive Na^+ influx was 4.5 ± 2.3 nmol · mg protein^{-1} · min^{-1} in cells exposed to high glucose and 5.2 ± 2.7 nmol · mg protein^{-1} · min^{-1} in control cells ($p = 0.05$). Hence, the decrease in total Na^+ influx observed in high-glucose exposed cells is attributable to decreased activity of the DMA-inhibitable Na^+/H^+ exchanger.

The effects of acute exposure to high glucose and chronic hypertonicity were investigated in four of the primary isolates whose response to chronic high glucose is presented in Figure 1. In these experiments, Na^+ influx was compared in cells exposed to control medium, high glucose for 8 ± 1.5 days or 24 h, and ribitol (25 mmol/l) for 8 ± 1.5 days (Fig. 2). Only in the cultures exposed to chronic high glucose was Na^+ influx different from control ($p < 0.05$). Protein content was similar in the four groups.

Because a decreased activity of the antiporter in cells chronically exposed to high glucose could reflect an altered intracellular acid-base balance, we measured the pHi. In cells attached to coverslips the resting pHi was 7.23 ± 0.1, ant it was not altered by culture in high glucose (7.24 ± 0.1). The protocol used to activate the antiporter (30 min of Na^+-free buffer with addition of ouabain and bumetanide in the last 5 min) lowered the pHi to the same extent in four isolates cultured in high glucose (6.92 ± 0.19) and in paired control cells (6.98 ± 0.15). Identical results were obtained when the pHi was measured in cell suspensions: the resting pHi was 7.23 ± 0.04 and after acidification it was 6.95 ± 0.08 both in cells cultured in high glucose and in control cells.

Northern blot analysis of the human endothelial cell Na^+/H^+ exchanger revealed a single transcript with a molecular size of 5.1 kilobases (Fig. 3), in agreement with the size reported for the exchanger of human promyelocytic HL-60 cells and rat aortic smooth muscle cells [25]. The levels of Na^+/H^+ exchanger mRNA in three pools of poly (A^+) prepared from first-passage cultures exposed to high glucose were 105 ± 40 % of control (Fig. 3). In the same cultures the levels of fibronectin mRNA were 160 % of control. Fibronectin expression was also measured in 6 of the 15 first-passage cultures tested for Na^+/H^+ exchanger activity (Table 1). Cultures exposed to high glucose and showing decreased Na^+ influx (86 ± 8 % of paired controls, $p = 0.03$) manifested instead up-regulation of fibronectin expression (151 ± 24 % of paired controls, $p = 0.03$). The actin mRNA levels in high glucose cultures were 100 ± 18 % of control.

Discussion

This study documents that exposure of human vascular endothelial cells to high ambient glucose for sev-

Table 1. Effects of high (30 mmol/l) glucose on Na^+/H^+ exchanger activity and fibronectin expression in human umbilical vein endothelial cells

Isolate	Na^+ influx			Fibronectin mRNA
	N (nmol · mg protein^{-1} · min^{-1})	H	H (% of N)	H (% of N)
1	9.3	6.6	71	133
2	12.9	11.5	89	130
3	10.8	9.2	85	181
4	13.7	12.6	92	126
5	9.2	8.6	93	162
6	12.0	10.7	89	175
Mean	11.3	9.8	86	151
SD	1.8	2.1	8	24

N, Cells grown in normal glucose; H, cells grown in high glucose

eral days results in a modest but consistent decrease in the activity of the Na^+/H^+ exchanger without obvious alterations in exchanger mRNA levels. The reduced antiporter activity cannot be attributed to a lesser stimulus in cells exposed to high glucose, insofar as the degree of intracellular acidification achieved in these cells was equal to, or greater than, that achieved in control cells.

Interpretation of the discrepant effect of high glucose on exchanger activity and mRNA levels must address the possibility that antiport function might not be subserved solely by the isoform for which the mRNA was measured. We studied the mRNA encoding the Na^+/H^+ exchanger isoform 1 referred to as NHE-1 [26], but at least three other isoforms have been identified and sequenced [26]. However, both NHE-2 and NHE-3 are resistant to DMA inhibition and, together with NHE-4, show a tissue distribution restricted to the gastrointestinal tract and kidney, possibly in connection with specific absorptive processes [26]. Hence, in the absence of reasons to anticipate that human umbilical vein endothelial cells may be endowed with exchanger isoforms other than the ubiquitous housekeeping NHE-1, it can be proposed that the effects of high glucose are exerted at the translational or post-translational level. Translational effects of high glucose cannot be excluded, but we have detected none to date in endothelial cells [7, 27], and the known mechanisms of antiporter activation are either pre- [25] or post-translational [24, 26].

Because the effects of high glucose were not mimicked by hypertonicity, post-translational modulation would be expected to occur through mechanisms uniquely set in motion by the glucose molecule. One such mechanism could be activation of protein kinase C (PKC) which can be induced by high glucose [12]. The C-terminus regulatory domain of NHE-1 has a PKC consensus sequence [26] and the reduction in exchanger activity measured in our cells after several days exposure to high glucose could be interpreted as reflecting PKC down-regulation. On the other hand, we have no evidence for PKC activation in human endothelial cells exposed to high glucose [13], and the unaltered exchanger activity after 24 h of high glucose does not support the occurrence of early activation to be followed by downregulation. Our findings differ from those of Williams and Howard [28] who reported recently that rat aortic smooth muscle cells exposed for 3–24 h to high glucose exhibit increased Na^+/H^+ exchanger activity and mRNA levels sustained by activation of PKC. Whether these differences in PKC sensitivity to high glucose reflect differences in cell type (endothelial vs smooth muscle) or species (human vs rat) will need to be clarified. The occurrence of reduced exchanger activity in our cells only after several days of exposure to high glucose would be compatible with an effect of non-enzymatic glycosylation on a critical binding domain of the Na^+/H^+ exchanger.

Alternatively, the endothelial cells Na^+/H^+ exchanger may be modulated by high glucose in a less immediate way, as a consequence of the excess synthesis and deposition [7] of extracellular matrix. The extracellular matrix can impart to anchorage-dependent cells mitogenic signals and the signalling pathway involves activation of the Na^+/H^+ exchanger [5]. There is, however, evidence that in the presence of excess matrix the proliferation of endothelial [29, 30] and other cells [31] is reduced. These observations, combined with the present finding that in cells overproducing matrix the response of the antiporter to mild intracellular acidification is decreased, indicate the need to examine whether concentrations of extracellular matrix that are growth-inhibitory do lead to reduced Na^+/H^+ antiporter responsiveness to other types of stimuli and, in particular, to mitogens. Support for this possibility is also offered by the recent report that renal tubular cells that are growth-inhibited by collagen IV substratum, also manifest decreased Na^+/H^+ exchanger activity [32]. It should be noted that a poorly proliferating phenotype is not necessarily caused or accompanied by decreased activity of the Na^+/H^+ exchanger; in fact in vascular smooth muscle cells perturbations resulting in decreased cellular proliferation (i.e. glucocorticoids) increase Na^+/H^+ exchanger activity [33]. This further suggests that the reduced antiporter activity observed in our system results from events specifically triggered by high glucose, and may causally contribute to the hampered replication of endothelial cells cultured in high glucose.

Two observations made in this study may be relevant to the ongoing attempt to clarify the origin and role of the elevated Na^+/H^+ exchanger activity in patients with diabetic renal disease. The first is that in the only instance in which chronic exposure to high glucose of human cells has been examined, the activity of the antiporter has been noted to change in a direction opposite to that recorded in some cells of dia-

betic patients with nephropathy. If the finding extends to the cell types usually studied in patients (fibroblasts, leucocytes), exposure to hyperglycaemia will not need to be considered among contributors to an elevated Na^+/H^+ antiporter activity. The second observation is that the mechanism of extracellular matrix accumulation – considered central to the development of diabetic kidney disease [34, 35] – is activated by high glucose at the same time as the function of the Na^+/H^+ exchanger is actually decreased compared to normal. On this basis it could be surmised that effects of high glucose on mesangial matrix accumulation [36] may occur irrespective of the level of exchanger activity, and that in diabetic patients hyperglycaemia and increased antiporter activity may impact on different processes involved in the development of nephropathy. This possibility is in agreement with the observations that approximately 40 % of insulin-dependent diabetic patients who develop kidney disease do not have elevated Na^+/Li^+ countertransport [37], and that the latter appears to increase predisposition to renal disease principally in patients with poor glycaemic control [37, 38].

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