

# GLUT2 protein at the rat proximal tubule brush border membrane correlates with protein kinase C (PKC)- $\beta$ I and plasma glucose concentration

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Received: 1 April 2007 / Accepted: 21 June 2007 / Published online: 11 August 2007  
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## Abstract

**Aims/hypothesis** GLUT2 is the main renal glucose transporter upregulated by hyperglycaemia, when it becomes detectable at the brush border membrane (BBM). Since glucose-induced protein kinase C (PKC) activation in the kidney is linked to diabetic nephropathy, we investigated the effect of glycaemic status on the protein levels of PKC isoforms  $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\delta$  and  $\epsilon$  in the proximal tubule, as well as the relationship between them and changes in GLUT2 production at the BBM. **Methods** Plasma glucose concentrations were modulated in rats by treatment with nicotinamide 15 min prior to induction of diabetes with streptozotocin. Levels of GLUT2 protein and PKC isoforms in BBM were measured by western blotting. Additionally, the role of calcium signalling and PKC activation on facilitative glucose transport was examined by measuring glucose uptake in BBM vesicles prepared from proximal tubules that had been incubated either with thapsigargin, which increases cytosolic calcium, or with the PKC activator phorbol 12-myristate,13-acetate (PMA).

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**Results** Thapsigargin and PMA enhanced GLUT-mediated glucose uptake, but had no effect on sodium-dependent glucose transport. Diabetes significantly increased the protein levels of GLUT2 and PKC- $\beta$ I at the BBM. Levels of GLUT2 and PKC- $\beta$ I correlated positively with plasma glucose concentration. Diabetes had no effect on BBM levels of  $\alpha$ ,  $\beta$ II,  $\delta$  or  $\epsilon$  isoforms of PKC.

**Conclusions/interpretation** Enhanced GLUT2-mediated glucose transport across the proximal tubule BBM during diabetic hyperglycaemia is closely associated with increased PKC- $\beta$ I. Thus, altered levels of GLUT2 and PKC- $\beta$ I proteins in the BBM may be important factors in the pathogenic processes underlying diabetic renal injury.

**Keywords** Brush border membrane · Diabetes · Facilitated glucose transporters · GLUT2 · Hyperglycaemia · Protein kinase C · Proximal tubule · Streptozotocin

## Abbreviations

BBM	brush border membrane
$K_t$	glucose concentration at half $V_{max}$
PBS-T	PBS containing 0.1% Tween 20
PKC	protein kinase C
PMA	phorbol 12-myristate,13-acetate
SGLT	sodium/glucose cotransporter
STZ	streptozotocin
$V_{max}$	maximum transport capacity

## Introduction

In the past decade significant advances have been made in defining the cellular mechanisms involved in diabetes-induced renal injury. Data from clinical trials and experimental models of diabetes have shown a strong correlation

between hyperglycaemia and the development and progression of diabetic nephropathy [1]. Most studies have concentrated on the effect of high glucose concentrations on mesangial cells, since glomerulopathy is the most characteristic clinical and histological feature of diabetic renal disease. These studies have provided convincing evidence that overproduction of GLUT1 and protein kinase C (PKC)- $\beta$ I are linked to glucose-induced mesangial cell damage and extracellular matrix deposition [1, 2]. Diabetes also increases renal size, mainly due to proximal tubular cell hypertrophy and thickening of the tubular cell basement membrane [3], leading to eventual tubular cell loss and declining renal function [4]; however, the underlying mechanisms affecting proximal tubular cell function and their relationship to glucose transport have not been studied in any detail.

In the last 5 years, the conventional model of glucose transport across renal and intestinal epithelia has been challenged. It is now clear that sodium/glucose cotransporters (SGLTs) and GLUT2 work in conjunction to transport glucose across the brush border membrane (BBM) over a wide range of glucose concentrations. The proposed model for enterocyte glucose uptake is that a high level of luminal glucose leads to rapid insertion of GLUT2 into the BBM via a calcium- and PKC- $\beta$ II-dependent mechanism [5–8]. There is less evidence to support a similar process occurring in the proximal tubule BBM, but because the basic glucose transport mechanisms are similar in both tissues, it seems likely that the same model may apply. Indeed, we have demonstrated that GLUT2 is translocated to the proximal tubule BBM in streptozotocin (STZ)-induced diabetes and that overnight fasting, which reduces plasma glucose concentrations to normal levels, reverses the BBM targeting of GLUT2 [9].

Previous studies have shown that many PKC isoforms are present in rat and mouse kidneys. Immunohistochemistry has localised different isoforms to specific nephron segments, and there are also distinct species differences. In rat kidney, the PKC isoforms  $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\delta$ ,  $\epsilon$  and  $\zeta$  have been detected in the proximal tubule [10] with prominent staining for PKC- $\alpha$  [11, 12] and - $\beta$ I [12] at the BBM. In contrast, PKC- $\alpha$  and - $\beta$  isoforms are undetectable in mouse proximal tubules, with PKC- $\epsilon$  being the predominant isoform in this species [13]. Studies have also detected altered levels of PKC isoforms in proximal tubular cells following exposure to high glucose concentrations, e.g. PKC- $\alpha$ , - $\epsilon$  [10] and - $\beta$ I [14] specifically were found to be increased. These findings suggest that diabetes-induced alterations in the levels and activity of PKC isoforms may affect proximal tubular cell function, as well as having their already established effects on glomerular cells.

Since GLUT1 and PKC- $\beta$ I may influence mesangial cell size in diabetes [1], and also due to the fact that PKC- $\beta$ II regulates GLUT2 protein levels at the enterocyte BBM, we

investigated the relationship between changes in circulating glucose concentration and protein levels of PKC- $\beta$ I, PKC- $\beta$ II and other PKC isoforms, together with GLUT2 abundance at the proximal tubule BBM. In order to study as wide a range of glucose concentrations as possible, we used normal animals and STZ-diabetic animals, with or without prior treatment with low or high doses of nicotinamide. Nicotinamide protects pancreatic beta cells from STZ cytotoxicity [15] and makes it possible to modulate blood glucose concentrations more effectively than by using graded doses of STZ.

## Methods

**Animals** The present study used 250 g male Sprague–Dawley rats bred in the Comparative Biology Unit at the Royal Free and University College Medical School. Two weeks prior to use animals received a single i.p. injection of nicotinamide dissolved in sterile saline (0, 50 and 200 mg/kg), followed 15 min later by a single tail vein injection of STZ (60 mg/kg) dissolved in freshly prepared citrate buffer (pH 4.5) under halothane anaesthesia (2% halothane in 100% oxygen). Animals were allowed free access to water and a standard rat chow (Diet RM1; SDS, Witham, Essex, UK) until the time of experimentation, except for those which were fasted overnight prior to killing. All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Plasma glucose concentrations were determined using a glucose oxidase method.

**Brush border membrane vesicle preparation** The methods used to prepare renal BBM vesicles have been described previously [9]. In brief, animals were anaesthetised with an i.p. injection of 60 mg/kg pentobarbitone sodium (Pentobarbitone, Animalcare, York, UK) and both kidneys removed and placed in ice-cold 154 mmol/l NaCl. After removal of the renal capsule, the kidneys were weighed, sliced into 2 mm sections and the cortex dissected away. Cortical fragments were suspended in 30 ml of buffer containing (mmol/l) 300 mannitol, 12 Tris HCl, 5 EGTA, pH 7.4. The resulting suspension was homogenised for 2 min (Ultra Turrax homogeniser; Janke & Kunkel, IKA Labortechnik, Staufen, Germany) at half speed, followed by the addition of 42 ml of cold distilled water and MgCl<sub>2</sub> to a concentration of 12 mmol/l; it was then stirred on ice for 15 min. The solution was then centrifuged for 15 min at 2,000 $\times$ g and the supernatant fraction re-centrifuged for 30 min at 21,000 $\times$ g. The pellet was suspended in 20 ml buffer containing (mmol/l): 150 mannitol, 6 Tris HCl, 2.5 EGTA (pH 7.4) using ten cycles of a hand-operated glass–Teflon homogeniser. MgCl<sub>2</sub> was added to a concentration of 12 mmol/l and, after stirring on ice for 15 min, the low and high-speed centrifugations described above were repeated.

The pellet was then resuspended in 20 ml buffer containing (mmol/l) 300 mannitol, 12 Tris HCl, 2.5 EGTA (pH 7.4) and centrifuged at  $21,000\times g$  for 30 min. The purified BBM pellet was finally suspended in the same buffer to a protein concentration of 3 to 6 mg/ml using five to six passes through a syringe fitted with a 21-gauge needle. All steps were carried out at 4°C. The concentration of protein and activity of alkaline phosphatase in the initial homogenate and BBM preparation were determined to derive values for enrichment of this marker enzyme.

*Proximal tubule exposure to thapsigargin and phorbol 12-myristate,13-acetate* Cortical fragments (prepared as above) from six kidneys were divided into two equal samples and were suspended in 30 ml buffer containing (mmol/l) 300 mannitol, 12 Tris HCl, 5 EGTA, pH 7.4. A suspension was then obtained by subjecting the solution to six strokes in a hand-held glass–Teflon homogeniser. Paired incubations were carried out by adding 1  $\mu\text{mol/l}$  phorbol 12-myristate,13-acetate (PMA) or 1  $\mu\text{mol/l}$  thapsigargin dissolved in DMSO to one-half of the suspension, while the other half received an equal volume of DMSO. Suspensions were incubated at 37°C in a shaking water-bath, while being gassed with 100% oxygen. After 30 min, tubules were separated by centrifugation (2 min at  $40\times g$ ) and BBM vesicles were prepared from the precipitate as described above.

*Brush border membrane uptake studies* Uptake studies were carried out at 20°C on freshly prepared vesicles as described for intestinal BBM [16]. In brief, the transport process was initiated by mixing equal volumes of vesicle suspension and uptake buffer consisting of (in mmol/l) 200 NaSCN, 20 HEPES, 0.1 MgSO<sub>4</sub> containing D-[<sup>3</sup>H]glucose, such that the final concentration of glucose was 30 to 960  $\mu\text{mol/l}$ . Uptake was terminated after 4 s by the addition of 3 ml of 154 mmol/l NaCl containing 0.5 mmol/l phlorizin maintained at 4°C, followed by vacuum filtration through 0.45  $\mu\text{m}$  nitrocellulose filters (Sartorius, Göttingen, Germany). Three further washes were carried out. Parallel uptakes using buffer containing 1 mmol/l phlorizin to block SGLT-mediated transport indicated that uptake was >96% phlorizin-sensitive. In order to assess GLUT-mediated transport, a higher glucose concentration (20 mmol/l) was used in the presence of 1 mmol/l phlorizin, in keeping with the low affinity of GLUT transporters for glucose binding [17]. From scintillation counting of the filters, BBM glucose accumulation was calculated and expressed as nmol mg protein<sup>-1</sup> 4 s<sup>-1</sup>. The kinetic parameters of maximum transport capacity ( $V_{\text{max}}$ ) and glucose concentration at half  $V_{\text{max}}$  ( $K_t$ ) for phlorizin-sensitive uptake were derived using a non-linear least squares curve-fitting program (GraphPad Software, San Diego, CA, USA).

*Western blotting* Polyclonal antibodies for GLUT2 and the PKC isoforms ( $\alpha$ ,  $\beta$ I,  $\beta$ II,  $\delta$  and  $\epsilon$ ) were purchased from Biogenesis (Poole, UK) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. For western blotting, BBM samples (15  $\mu\text{g}$  protein) were solubilised in Laemmli sample buffer containing 5% SDS and 5% mercaptoethanol, and electrophoresed on a 10% SDS polyacrylamide gel. The proteins were transferred to nitrocellulose membranes by semi-dry electrophoretic blotting for 1 h at a constant current of 1 mA/cm<sup>2</sup>. Non-specific protein-binding sites were blocked with PBS containing 0.1% Tween 20 (PBS-T) and 5% fat-free milk for 1 h at room temperature. The membranes were incubated with GLUT2 (1:1000), PKC- $\alpha$  (1:400), PKC- $\beta$ I (1:200), PKC- $\beta$ II (1:200), PKC- $\delta$  (1:500) and PKC- $\epsilon$  (1:500) antibodies for 16 h at 4°C. The filters were then washed (2 $\times$ 15 min) with PBS-T containing 1% fat-free milk and incubated for 2 h at room temperature with either anti-rabbit (1:2,000) (Amersham Pharmacia Biotech UK, Little Chalfont, UK) or anti-mouse (1:5,000) (Sigma, Poole, Dorset, UK) IgG antibody conjugated to horseradish peroxidase and finally washed again with PBS-T. Bound antibodies were detected using a western blot detection kit (Visualizer; Upstate Cell Signaling Solutions, Dundee, UK) and visualised and quantified using a Fluor-S MultiImager System (Biorad, Hemel Hempstead, UK). The abundance of GLUT2 relative to total protein concentration was established for each sample and expressed as a percentage of the control.

*Statistics* Data are presented as mean $\pm$ SEM of six animals per experimental group. Normal distribution was determined by Kolmogorov–Smirnov test of normality and statistical comparisons made using either an unpaired *t* test or a one-way ANOVA with post hoc comparisons performed using either the Bonferroni multiple comparisons test or the Kruskal–Wallis test. All analyses were performed using Graphpad InStat software (GraphPad Software) with statistical significance taken as  $p<0.05$ .

## Results

Animals treated with STZ alone gained less weight over the 2 week experimental period than control animals. In contrast, animals receiving 50 or 200 mg/kg nicotinamide prior to STZ gained weight at the same rate as control animals (Table 1). Kidney weight was significantly increased following STZ injection, but not when animals were pretreated with nicotinamide (Table 1). Animals receiving STZ alone showed a 2.8-fold increase in plasma glucose concentration compared with control (Fig. 1); those pretreated with low dose nicotinamide (50 mg/kg) had

**Table 1** Effect of STZ and nicotinamide on animal weight gain, kidney weight and enrichment of alkaline phosphatase in BBM vesicles

Parameter	Control	STZ	STZ + 50 mg/kg nicotinamide	STZ + 200 mg/kg nicotinamide
Weight gain (g)	63.3±8.1	24.0±6.1*	57.3±5.85	59.5±4.37
Kidney weight (mg kidney/g body weight)	3.95±0.16	5.24±0.1***	4.00±0.18	3.63±0.08
BBM enrichment	11.35±0.65	13.09±0.45	12.3±0.99	11.68±0.7

Values are mean±SEM ( $n=12-18$ )

\* $p<0.05$ , \*\*\* $p<0.001$  compared with control using ANOVA with Tukey–Kramer post hoc test

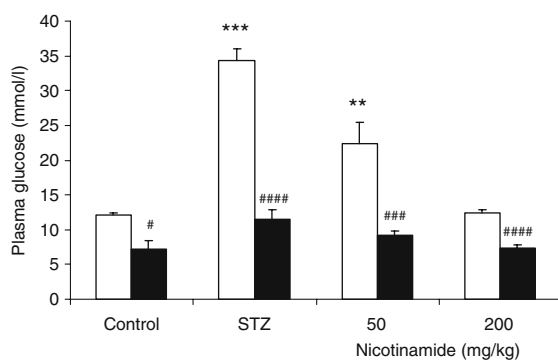
plasma glucose levels 1.85-fold greater than controls, whereas high dose nicotinamide (200 mg/kg) resulted in plasma glucose levels similar to those of controls. Overnight fasting of each group also reduced plasma glucose concentrations to levels similar to those of non-fasted controls (Fig. 1).

BBM vesicles used for western blotting and uptake studies were enriched with the BBM marker enzyme alkaline phosphatase (Table 1). Treatment with STZ or nicotinamide had no significant effect on enrichment.

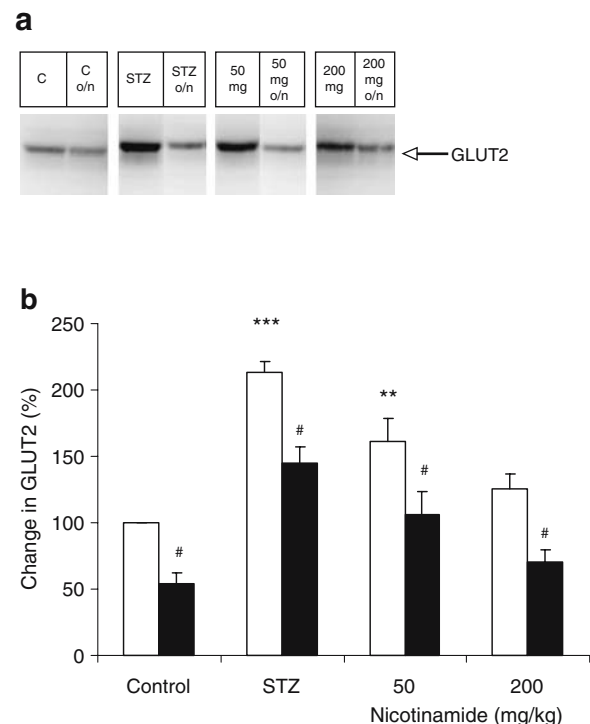
We have reported previously that STZ-induced diabetes results in translocation of GLUT2 protein to the BBM and that this is likely to be due to hyperglycaemia [9]. The present study aimed to determine whether more modest changes in plasma glucose concentration had any effect on GLUT2 levels at the BBM. Western blotting for GLUT2 protein in BBM vesicles prepared from animals treated with STZ alone and from those pretreated with 50 mg/kg nicotinamide revealed an increase in GLUT2 (Fig. 2). In STZ animals pretreated with 200 mg/kg nicotinamide, GLUT2 levels were similar to non-diabetic levels before and after overnight fasting.

Recent studies have described the involvement of PKC and calcium-dependent signalling pathways in the control of facilitative glucose transport in mesangial cells and intestinal

enterocytes [1, 6, 18]. In the present study, incubation of proximal tubules for 30 min with 1  $\mu\text{mol/l}$  PMA, a PKC agonist, had no effect on the  $V_{\text{max}}$  or the  $K_t$  (control:  $505\pm54$  vs PMA:  $462\pm18$   $\mu\text{mol/l}$ ) of SGLT-mediated glucose transport (Fig. 3). In contrast, GLUT-mediated uptake was significantly enhanced by PMA (Fig. 3). Similar responses were observed following exposure of proximal tubules to 1  $\mu\text{mol/l}$  thapsigargin (which releases  $\text{Ca}^{2+}$  from cellular stores): the  $V_{\text{max}}$  and  $K_t$  for SGLT-mediated uptake were unaffected ( $K_t$  control:  $431\pm47$  vs thapsigargin:  $435\pm$



**Fig. 1** Effect of STZ and nicotinamide on plasma glucose levels in non-fasted (*open bars*) and overnight fasted (*closed bars*) animals. Results are expressed as mean±SEM,  $n=6-12$ . \*\* $p<0.01$ , \*\*\* $p<0.001$  compared with non-fasted control animals using ANOVA with Tukey–Kramer post hoc test; # $p<0.05$ , ### $p<0.001$ , #### $p<0.0001$  compared with non-fasted animals using unpaired  $t$  test



**Fig. 2** **a** Representative western blot of GLUT2 in BBM vesicles of control and STZ-injected animals, with or without treatment with nicotinamide (centre right and right: 50 or 200 mg) or overnight (*o/n*) fasting. **b** Quantification of GLUT2 in BBM vesicles of non-fasted (*open bars*) and overnight fasted (*closed bars*) animals in the different experimental groups. Results are expressed as a percentage of GLUT2 compared with control,  $n=6-12$ . \*\* $p<0.01$ , \*\*\* $p<0.001$  compared with non-fasted control group using ANOVA with Dunn's multiple comparison post hoc test; # $p<0.05$  compared with non-fasted animals using unpaired  $t$  test

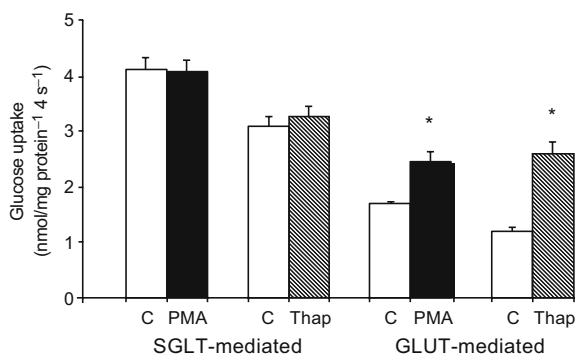
16  $\mu\text{mol/l}$ ), whereas GLUT-mediated glucose uptake was significantly enhanced (Fig. 3).

Using western blotting, we examined the effects of STZ-diabetes on levels of PKC- $\alpha$ , - $\beta$ I, - $\beta$ II, - $\delta$  and - $\epsilon$  protein in BBM vesicles. Diabetes had no effect on the levels of PKC- $\beta$ II,  $\alpha$ ,  $\delta$  or  $\epsilon$ , but levels of PKC- $\beta$ I were significantly increased (Fig. 4). We then examined the effect of smaller variations in blood glucose concentration on BBM levels of PKC- $\beta$ I. This was achieved by administering nicotinamide 15 min prior to STZ and by overnight (18 h) fasting of both non-diabetic and diabetic animals. Figure 5 shows that treatment with 50 and 200 mg/kg nicotinamide reduced PKC- $\beta$ I, while overnight fasting in all the groups decreased the protein to the levels found in control animals.

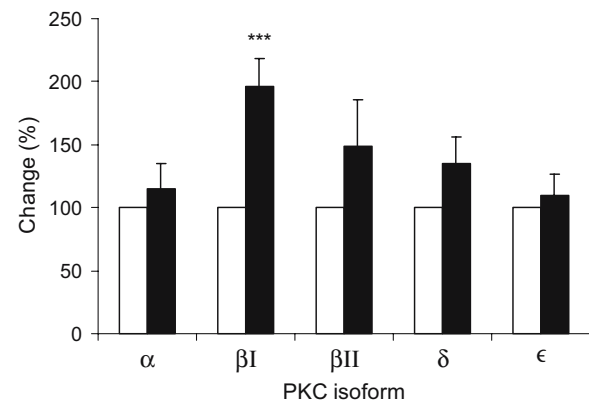
The relationship between plasma glucose concentration, GLUT2 and PKC- $\beta$ I was also examined by cross-correlating these three variables. It is clear that over the range of plasma glucose concentrations recorded, GLUT2 protein levels correlated positively with plasma glucose concentration ( $r=0.718$ ,  $p<0.0001$ ) (Fig. 6a). Similarly, PKC- $\beta$ I also correlated significantly with plasma glucose concentration ( $r=0.779$ ,  $p<0.0001$ ) (Fig. 6b). The correlation of BBM levels of GLUT2 with PKC- $\beta$ I gave an  $r$  value of 0.648 ( $p<0.0001$ ) (Fig. 6c).

## Discussion

The present study shows clearly that changes in circulating glucose concentration can rapidly influence the levels of GLUT2 protein at the proximal tubule BBM. GLUT-mediated transport activity at the BBM was also affected by activation of PKC and by increased cytosolic calcium. Protein levels of the calcium-dependent PKC isoform, PKC- $\beta$ I, correlated closely with the increase in GLUT2 and with plasma glucose concentration.

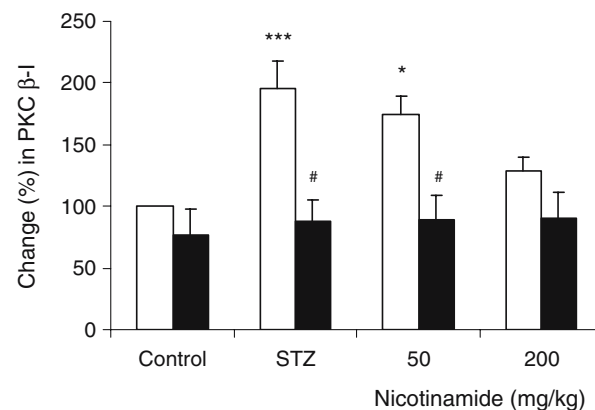


**Fig. 3** Effect of PMA and thapsigargin on BBM vesicle glucose transport.  $V_{\text{max}}$  of SGLT-mediated transport was determined from uptakes measured at 30–960  $\mu\text{mol/l}$  glucose and GLUT-mediated uptake was measured at 20  $\text{mmol/l}$  glucose. Values are expressed as mean  $\pm$  SEM of six vesicle preparations. \* $p<0.05$  compared with control using paired  $t$  test. C Control, Thap thapsigargin

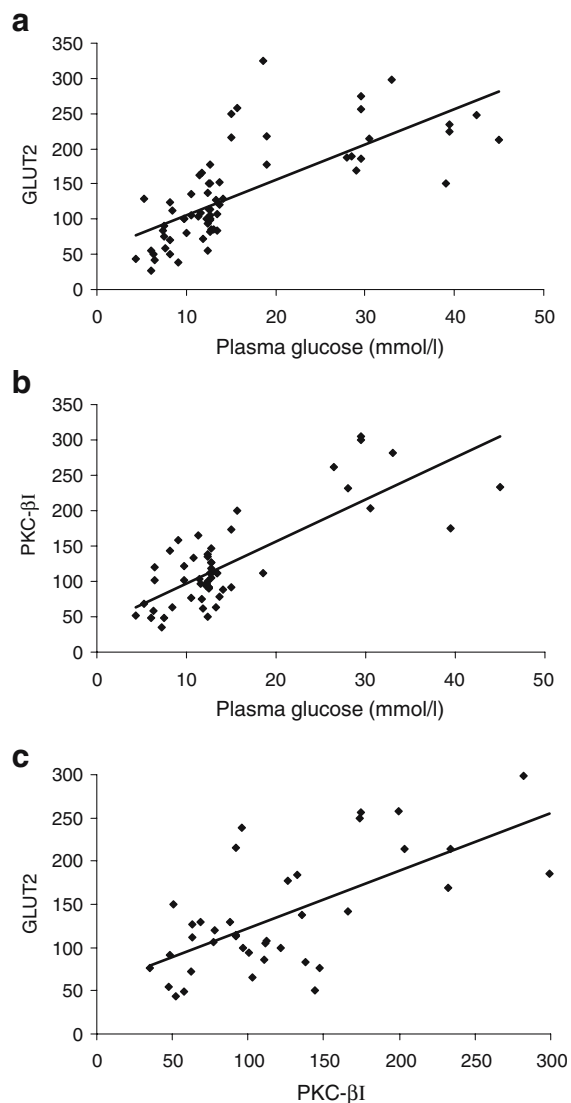


**Fig. 4** Quantification of PKC isoforms in BBM vesicles of control (open bars) and STZ-treated (closed bars) animals. Results are expressed as a percentage compared with control,  $n=6-12$ . \*\*\* $p<0.001$  compared with control using unpaired  $t$  test

These findings are relevant to our understanding of the processes underlying diabetes-related changes in proximal tubular cell function. Although most studies have concentrated on the mechanisms of glucose-induced glomerular damage, there is evidence that tubular cell hypertrophy and basement membrane thickening are among the earliest pathological changes occurring in diabetes [19]. Tubulointerstitial fibrosis in diabetes is characterised by accumulation of extracellular matrix proteins, including fibronectin and collagen, leading eventually to tubulointerstitial scarring [20] and loss of tubular cell function. Unlike the glomerulus, exposure of human proximal tubular cells to elevated glucose levels does not affect collagen or fibronectin gene expression, but does result in a change in their degradation and rate of turnover [3, 21]. The tubulointerstitium comprises much of the kidney substance, and tubular hypertrophy is responsible for most of the renal



**Fig. 5** Quantification of PKC- $\beta$ I in BBM vesicles of control and STZ-injected animals, with or without treatment with nicotinamide (open bars) or overnight (closed bars) fasting. Results are expressed as percentage change in PKC- $\beta$ I expression compared with control,  $n=6-12$ , \* $p<0.05$ , \*\*\* $p<0.001$  compared with non-fasted control animals using Kruskal–Wallis ANOVA; # $p<0.05$  compared with non-fasted animals using unpaired  $t$  test



**Fig. 6** Pearson's correlation between **a** GLUT2 protein levels and plasma glucose concentration, **b** PKC- $\beta$ I protein levels and plasma glucose concentration and **c** GLUT2 and PKC- $\beta$ I protein levels.  $r=0.718$  (**a**),  $r=0.779$  (**b**),  $r=0.648$  (**c**);  $p<0.0001$  (**a–c**)

enlargement seen in diabetes [4]. Although the transport mechanisms responsible for the increase in tubular glucose transport during diabetes (and therefore potentially the associated tubular hypertrophy) have been extensively studied, they have yet to be fully defined.

Studies focusing on the effect of diabetes on renal SGLT-mediated glucose transport have yielded conflicting results, with reports of both increased [22] and decreased [23] rates of transport. Moreover, our previous study demonstrated that in STZ-induced diabetes the sodium-dependent component of renal glucose transport was unchanged, whereas the facilitative (GLUT-mediated) component was significantly enhanced. Western blotting showed an increase in both GLUT2 and GLUT5 protein at the BBM, with no change in GLUT1 protein levels; and that the upregulation of GLUT2, but not

of GLUT5, could be abolished by overnight fasting [9]. Similar findings were reported by Freitas et al., who concluded that plasma glucose concentration was the key regulator of the gene encoding GLUT2 [24].

Our present results demonstrate that a reduction in plasma glucose concentration in STZ-diabetic rats treated with nicotinamide reduces STZ-induced renal hypertrophy, as well as GLUT2 protein levels. Therefore, since GLUT5 is considered to be a fructose transporter in vivo, with only a low affinity for glucose [25], and GLUT1 protein levels in the renal tubule are generally considered to be reduced [26, 27] or unchanged in diabetes [9], it is reasonable to hypothesise that GLUT2 might be responsible for glucose-induced tubular cell hypertrophy in diabetes. Indeed, two indirect findings also suggest a potential pathophysiological link between GLUT2 function and the kidney: one is the finding of renal hypertrophy and glomerulopathy in patients with GLUT2 mutations in the Fanconi–Bickel syndrome [28]; the other is the recent observation that flavonoids, which are known to ameliorate the changes of diabetic nephropathy in experimental models [29], inhibit GLUT2 production [30]. It is intriguing to speculate whether the beneficial effects of flavonoids in diabetes may be related, in part, to an inhibitory effect on GLUT2 in the kidney (and small intestine). Moreover, the DCCT showed the importance of tight control of blood glucose levels in preventing or delaying the onset of diabetic nephropathy [31], a finding with which our own may be consistent, since even modest elevations in plasma glucose concentration can affect GLUT2 levels at the BBM.

There is growing evidence that targeting SGLT-mediated renal glucose transport using phlorizin-like inhibitors may be useful in treating diabetes [32]. Two sodium-dependent transporters are produced in the kidney: the bulk of the filtered glucose is reabsorbed in the S1 segment by the low-affinity, high-capacity transporter, SGLT2; whereas the high-affinity, low-capacity transporter, SGLT1, scavenges the remaining glucose more distally (reviewed in [17]). However, the predicted  $K_m$  values for these two transporters are 0.5 and 2 mmol/l for SGLT1 and SGLT2, respectively, and in the setting of a high filtered glucose concentration, as in uncontrolled diabetes, both transporters would be saturated. Therefore, GLUT2 in the kidney may be unregulated, as occurs in the small intestine [33], where high glucose concentrations 'sensed' by SGLT proteins lead to rapid insertion of GLUT2 into the BBM. With a predicted  $K_m$  of 20 to 40 mmol/l [17], GLUT2 would provide a capacity for glucose absorption that is much higher than that of the SGLT proteins. Thus, inhibitors of SGLT in diabetes may act by preventing this mechanism of GLUT2 upregulation and BBM targeting.

Substantial evidence now exists for a role of GLUT2 in the transport of glucose across the BBM of renal and

intestinal epithelial cells. Although most studies have used the small intestine, the mechanisms controlling glucose uptake by proximal tubular cells display striking similarity to those occurring in enterocytes. In both cell types, activation of the protein kinase A signalling pathway increases intracellular cAMP levels and promotes SGLT1-mediated glucose transport [16, 34]. Short-term modulation of SGLT1-mediated transport occurs via apical membrane insertion of additional SGLT1 protein [35] and through changes in the membrane electrochemical gradient [16]. In enterocytes, activation of the PKC signalling pathway by PMA and the influx of  $\text{Ca}^{2+}$  through L-type channels have been shown to induce cytoskeletal re-arrangement, leading to insertion of GLUT2 protein at the BBM [6–8, 18, 36].

There is less information on the regulation of GLUT2 in the kidney; however, our finding that incubation of isolated proximal tubules with PMA and thapsigargin increases GLUT-mediated, but not sodium-dependent glucose transport indicates that, as in the small intestine, PKC and calcium are potential signalling pathways regulating renal BBM GLUT2 protein levels. It has been reported that basal levels of cytosolic calcium in proximal tubule cells are elevated in rats with STZ-induced diabetes [37] and that high extracellular glucose increases cytosolic calcium in cultured proximal tubular cells by activation of L-type  $\text{Ca}^{2+}$  channels [38]. However, our results highlight one noticeable difference between the kidney and intestine. Thus, although in the kidney numerous PKC isoforms are detectable at the proximal tubule BBM, increased GLUT2 protein at the BBM is correlated only with increases in PKC- $\beta$ I; in the small intestine, by contrast, there is a strong correlation between the levels of GLUT2 and PKC- $\beta$ II [6, 18, 36].

These differences in the PKC- $\beta$  isoforms responsible for regulation of GLUT2 in kidney and small intestine may be due to differential tissue production of these splice variants. In keeping with our findings, Hsieh et al. reported recently that glucose-induced damage to proximal tubule cells is dependent on PKC- $\beta$ I [14]. In addition, although there are conflicting results on which isoform(s) is(are) increased in the glomeruli of STZ-diabetic animals, with reports of increased levels of PKC- $\alpha$  [2, 10, 39], - $\beta$ I [2, 39, 40], - $\delta$  [41] and - $\epsilon$  [10, 41], PKC- $\beta$ I seems to be the pathophysiological isoform [40, 42]. Indeed, there is strong evidence that diabetes-induced mesangial cell damage occurs as a result of increased glucose uptake by GLUT1, which correlates with increased PKC- $\beta$ I activation and fibronectin and collagen accumulation [1]. Findings in the glomerulus, and our present findings, draw attention to the link between PKC activation and increased production of GLUT transporters in diabetes-induced renal damage.

Additional evidence that the PKC- $\beta$ I isoform is the predominant pathophysiological isoform in the kidney

comes from studies using PKC- $\beta$ -deficient (*PKC- $\beta$ <sup>-/-</sup>*) mice and the PKC- $\beta$  inhibitor, LY333531. Levels of the extracellular matrix proteins collagen and fibronectin, as well as those of the profibrotic cytokine TGF- $\beta$  are decreased in *PKC- $\beta$ <sup>-/-</sup>* mice; moreover, a significant reduction in renal hypertrophy and glomerular enlargement is seen when *PKC- $\beta$ <sup>-/-</sup>* mice are made diabetic and compared with wild-type mice [39, 43]. Feeding of LY333531 to animals with STZ-induced diabetes has also been shown to prevent the increases in extracellular matrix components like fibronectin and collagen [2] and to reduce albuminuria and structural injury to the glomerulus [40, 42, 44]. Interestingly, inhibition of PKC- $\beta$  by LY333531 also attenuated the diabetes-induced changes in the tubulointerstitium [40, 45]. Clinical trials with LY333531, which is well tolerated [46, 47], indicate that it decreases albuminuria and maintains GFR [48], and can reduce microvascular complications in patients with diabetes [49, 50]. Therefore, inhibition or lack of PKC- $\beta$ I production appears to protect against glucose-induced renal dysfunction and fibrosis.

In conclusion, we have demonstrated that raised circulating glucose levels promote targeting of GLUT2 protein to the proximal tubule BBM and that the levels of GLUT2 protein correlate with small changes in glucose concentration within the pathophysiological range. GLUT-mediated glucose uptake at the BBM is enhanced by PKC activation and an increase in cytosolic calcium; levels of GLUT2 and PKC- $\beta$ I in the BBM are also closely correlated. These findings highlight the potential significance of altered PKC- $\beta$ I and GLUT2 in diabetic renal tubulopathy.

**Acknowledgements** We are grateful to the St Peter's Trust of the Middlesex Hospital for financial support.

**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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