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GLUT2 protein is translocated to the rat proximal tubule brush-border membrane (BBM) in response to diabetic hyperglycaemia

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The conventional model of renal glucose reabsorption involves uptake from the proximal tubule lumen across the brush-border membrane (BBM) by a sodium-dependent transporter, SGLT, followed by exit across the basolateral membrane (BLM) via a facilitative, GLUT-mediated transporter. Our previous studies have shown that streptozotocin (STZ)-induced diabetes has no effect on SGLT-mediated transport but increases GLUT-mediated transport across the renal BBM (Debnam *et al.* 1997), which is associated with increased levels of GLUT2, demonstrated by Western blotting. However, immunohistochemistry performed on unfixed tissue sections failed to detect this protein at the BBM. The present study investigated whether kidney fixation *in vivo* yielded the same pattern of GLUT2 expression as that obtained in unfixed tissue.

Diabetes was induced in male Sprague-Dawley rats using a single tail vein injection of STZ (45 mg kg^{-1}). Three-week diabetic, overnight fasted diabetic and weight-matched control animals ($n = 4$ per experimental group) were terminally anaesthetised with pentobarbitone sodium (90 mg kg^{-1} I.P.) and the left kidney perfused with periodate-lysine-paraformaldehyde (PLP) (Mclean & Nakane, 1974). Fixed kidneys were removed, embedded in OCT and snap frozen in liquid N_2. Cryostat sections (7 μm) were mounted onto polysine-coated slides and a standard peroxidase-DAB immunohistochemical protocol used for the localisation of GLUT2 protein. Experiments were performed in accordance with the Animals (Scientific Procedures) Act, 1986.

Immunohistochemistry performed on kidneys fixed *in vivo* with PLP showed GLUT2 labelling in the S1 segment of the proximal convoluted tubule (PCT). In control kidneys, GLUT2 was localised exclusively to the BLM, whereas in diabetic kidneys it was detected at both the BLM and BBM. Overnight fasting of the diabetic animals reduced blood glucose levels and returned the distribution of GLUT2 immunoreactivity to that of controls.

In vivo fixation seems crucial in the detection of GLUT2 at the BBM of PCT cells in diabetes. GLUT2 protein appears to be rapidly shuttled in or out of the BBM in response to the glycaemic status of the animal. It is noteworthy that in intestinal enterocytes, where the transport process of glucose is very similar to that in the PCT, BBM levels of GLUT2 also increase in response to high luminal glucose concentrations (Kellet *et al.* 2000).

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All procedures accord with current UK legislation.

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The effect of P2 receptor inhibition on cultured MDCK cysts *in vitro*

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Although the genetic basis of autosomal dominant polycystic kidney disease (ADPKD) is known, the mechanism(s) of cyst formation and growth remains unclear. One possible mechanism is the activation of ATP-sensitive P2 receptors in the cells lining the cysts. Stimulation of a putative P2 receptor by ATP, acting in an autocrine or paracrine fashion, may stimulate Cl^- and fluid secretion and thus contribute to cyst expansion, as well as affect cell turnover, which can also affect cyst growth. In this study we investigated the effect of P2 receptor inhibition on cyst growth in a Madin Darby canine kidney (MDCK) cell culture model of renal cyst formation.

MDCK cells were cultured in collagen gels in the presence of the cAMP agonist forskolin to stimulate cyst formation, using a modification of the method of Grantham *et al.* (1989). Briefly, cells were suspended in vitrogen (~3.0 mg ml^{-1} collagen; Cohesion Technologies Inc., Palto Alto, USA), seeded onto 24-well plates and after gelation occurred, overlaid with growth media containing 10 μM forskolin. We tested the effects of three non-selective P2 receptor inhibitors: 100 μM suramin, 100 μM Pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonate (PPADS), and 100 μM Reactive blue 2 (RB2). For each treatment, $n = 40$–54 cysts. The diameter of each cyst was measured directly from photographs using images that had been magnified to the same extent.

Suramin and RB2 significantly reduced the increase in cyst volume by 39.45 % ($P = 0.001$; Student's paired t test) and 60.20 % ($P = < 0.001$), respectively. PPADS reduced the increase in cyst volume by 13.83 %, but this was not significant.

These findings support the hypothesis for a role of P2 receptors in renal cyst growth and enlargement. However, these initial observations require more study, since there are many P2 receptor subtypes, one or other of which might prove to be a therapeutic target to limit cyst growth and preserve renal function.

Grantham JJ *et al.* (1989). *Kidney Int* **35**, 1379–1389.

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Renal expression of the iron transporter DMT1 is reduced in streptozotocin-induced diabetic rat kidney

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The generation of reactive oxygen species (ROS) by metal oxidants such as iron, represents a potential damage mechanism in diabetic nephropathy and altered iron homeostasis is observed

in diabetic humans and animal models (Shah, 2001). If true, then the proteins responsible for iron uptake and handling could contribute to this process and their relative expression levels could be relevant in diabetic nephropathy. One such protein is the divalent metal transporter, DMT1, which we have previously shown to be differentially expressed along the nephron (Ferguson *et al.* 2001). Here, we report chronic down-regulation of DMT1 in the kidneys of diabetic rats.

Experiments were performed in accordance with the UK Animals (Scientific Procedures) Act and rats were killed humanely at the end of the experiments. Experiments were performed on two series of rats, Sprague-Dawley (series 1) and Wistar-Kyoto (series 2) (Charles River Laboratories, Kent). Rats were rendered diabetic with streptozotocin (STZ; Series 1, 60 mg kg^{-1} I.P. in citrate buffer; Series 2, 55 mg kg^{-1} I.P. in sterile saline). After 2 weeks, Series 1 rats were either killed for tissue collection or perfusion-fixed with 4% paraformaldehyde under anaesthetic (sodium thiopentone I.P.) for immunohistochemistry. For Series 2, animals were killed after 12 weeks of diabetes either in the absence or presence of (a) insulin replacement (interscapular subcutaneous 14% porcine insulin implant (Linplant; LinShin Canada Inc., Toronto, Canada) under halothane anaesthesia) or (b) treatment with the aldose reductase inhibitor, sorbinil (20 mg kg^{-1} day^{-1} P.O. for the final 5 weeks). Kidneys were collected and particulate (crude membrane) protein fractions prepared by differential ultracentrifugation. DMT1 expression was studied by semi-quantitative immunoblotting and immunofluorescence.

In Sprague-Dawley rats, renal DMT1 expression is reduced by $62 \pm 9\%$ (mean ± S.E.M.; $P < 0.01$ by unpaired t test; $n = 4$) following 2 weeks of STZ-diabetes. These changes were confirmed by immunofluorescence, which revealed a clear reduction in DMT1 expression from the proximal tubules to the collecting ducts. To exclude the possibility that DMT1 down-regulation is short-lived, we studied the renal DMT1 content of Wistar rats following 12 weeks of STZ-diabetes. Here, renal DMT1 expression was reduced by $34 \pm 4\%$ ($P < 0.01$ by ANOVA; $n = 5$), whereas in insulin-replaced diabetics, DMT1 expression was returned to control levels ($-10 \pm 7\%$; $P < 0.05$ *vs.* untreated diabetic, n.s. *vs.* non-diabetic; $n = 5$). Treatment with sorbinil for 5 weeks failed to significantly alter renal DMT1 expression in the STZ-diabetic Wistar rats, although a trend towards control was observed (Diab, $-41 \pm 2\%$; sorbinil/diabetic, $-19 \pm 5\%$, n.s., $n = 3$). We conclude that expression of the iron transporter DMT1 is significantly reduced in two strains of rat with chronic diabetes. Further work will be required to determine if renal DMT1 down-regulation contributes to iron-mediated damage in diabetic nephropathy, either by enhancing it or protecting against it.

Ferguson CJ *et al.* (2001). *Am J Physiol* (*Renal*) **280**, F803–814.
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