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A ROLE FOR UROEPITHELIAL GLUCOSE SIGNALLING IN DIABETIC BLADDER DYSFUNCTION

Hypothesis / aims of study

Diabetes mellitus (DM) is a metabolic disorder caused by dysregulation of cellular glucose-uptake, leading to hyperglycemia and excessive urine production (polyuria). Diabetic bladder dysfunction (DBD) is a prevalent complication of DM, characterized by a broad spectrum of symptoms including urinary urgency, frequency, and incontinence.

In non-insulin-dependent tissues such as the retina, lens and nerves, chronic elevation in cytosolic glucose leads to metabolic abnormalities including osmotic and oxidative stress, thought to be factors that contribute to tissue injury and dysfunction associated with long term DM. In addition, glucose per se is now known to act as a signaling molecule, modulating gene expression via the activation of the transcription factors, such as the recently identified carbohydrate response element binding protein (ChREBP) (1).

Using the streptozotocin-induced diabetic rat model (STZ-DM), it has recently been reported that the stratified epithelial lining of the urinary bladder (the uroepithelium) undergoes temporal changes in morphology with denuding of the highly differentiated surface (umbrella) cell layer, by 9 weeks post DM-induction. In addition, there was evidence of temporal modulation of uroepithelial gene expression/phenotype (2). As we have evidence that the uroepithelium expresses the non-insulin dependent glucose transporter-1 (GLUT1), these temporal changes may be as a direct result of increased cytosolic glucose due to exposure to elevated glucose on the serosal /blood side in addition to glucose in the urine.

The uroepithelium is both a barrier and a dynamic sensory tissue, expressing a wide array of receptor types and releasing neuroactive molecules such as adenosine triphosphate (ATP), nitric oxide (NO) and acetylcholine (ACh) (3). As such, it is likely to play an important role in bladder function by actively communicating with bladder nerves, smooth muscle and cells of the immune and inflammatory systems. Breach of the vital blood-urine barrier as well as changes in uroepithelial signaling in DM, may contribute to the symptoms of DBD.

This study was undertaken to investigate a direct impact of elevated glucose on temporal morphological and phenotypic changes of the uroepithelium noted in STZ-diabetic animals. The aim was to compare the temporal impact of polyuria under conditions of normoglycemia on uroepithelial structure and gene expression with existing data on the STZ-diabetic rat model (polyuria with hyperglycaemia) at 3 weeks and 9 weeks following initiation of polyuria. In addition, evidence of uroepithelial ChREBP expression and activity was examined *in vitro* using primary cultured uroepithelial (UT) cells.

Study design, materials and methods

Chronic non-osmotic polyuria was induced in adult (3 month old; n=8) female Sprague Dawley rats by addition of sucrose (5%) to drinking water, inducing polydipsia and polyuria without causing changes to plasma glucose levels (normoglycemia). Polyuria was confirmed using metabolic cages and normoglycemia was confirmed by blood sampling prior to sacrifice. Animals were humanely sacrificed at 3- and 9-weeks along with age-matched /untreated control rats (n=8). Bladders were weighed immediately upon excision and divided into sub-groups (n=4 per group) to be processed for molecular studies (reverse-transcription-quantitative polymerase chain reaction; RT-QPCR), and morphological studies using transmission and scanning electron microscopy. For molecular studies, excised urinary bladders were carefully cut open to expose the uroepithelial lining and pinned to a Sylgard-coated dish containing chilled, oxygenated Hank's balanced saline solution (HBSS). Using a dissecting microscope the mucosa was subsequently dissected from the underlying tissue and processed for reverse transcription as previously reported. Quantitative PCR (QPCR) amplification, normalized to the reference gene hypoxanthine-guanine phospho-ribosyltransferase (HPRT), compared temporal uroepithelial gene expression in test and control groups. Results were expressed as fold difference test versus control (Livak Method, $\Delta\Delta$Ct).

Cell Culture: Uroepithelial cells were isolated and grown in either normal/unmodified culture medium or medium containing elevated glucose (20mM).Fluorescent immunocytochemistry, using a ChREBP antibody was employed to investigate the uroepithelial expression of this transcription factor and response to elevated glucose. Control for specificity of the antibody was performed by pre-incubation with the ChREBP peptide.

Results

Bladders exhibited characteristic increase in mass in response to polyuria. TEM and SEM examination found no difference in morphology between test and control uroepithelium at both time points in contrast to findings in STZ-DM uroepithelium. Gene expression profile was dissimilar to observations in STZ-DM uroepithelium; this was noted as early as three weeks post induction of polyuria. In particular, GLUT1 was downregulated and there was no change in TRPV1 (transient receptor potential vanilloid subfamily member 1, proposed to act as a polymodal receptor and play a role in UT mechanosensation) expression in the 5% sucrose tissue at 3 weeks in contrast to STZ-DM findings of significant upregulation of both genes the same time point. Uroepithelial cells were found to express ChREBP, which exhibited a predominant nuclear location when cells were grown in elevated glucose (20mM).

Interpretation of results

Our morphological studies show no indication that polyuria per se (under conditions of normoglycemia) causes damage to the uroepithelial barrier in stark contrast to our findings at 9wk in STZ-DM bladders. These findings support our proposal that the uroepithelium is susceptible to metabolic stress under conditions of sustained hyperglycaemia, as seen in the STZ-DM rat model.

The novel finding of UT expression of the glucose sensing transcription factor, ChREBP, coupled with distinct differences in gene expression as early as 3 weeks between STZ-DM uroepithelium and uroepithelium from 5% sucrose polyuric animals, indicates the potential that glucose may act as a signalling molecule in the uroepithelium, modulating gene expression. Taken

together our findings suggest that in addition to inducing metabolic stress, glucose per se may cause changes in uroepithelial phenotype, resulting in modifications in sensory and signalling function, contributing from an early time point to the symptoms of diabetic bladder dysfunction.

Concluding message

The precise molecular mechanisms associated with DBD progression from onset of glucose dysregulation and polyuria to organ dysfunction remain elusive. Treatment modalities are limited and as the incidence of diabetes mellitus continues to rise, the importance and medical costs associated with this syndrome are likely to escalate. Therefore it is important to identify underlying mechanisms involved in the pathogenesis of DBD in order to develop new approaches to treatment.

References

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Disclosures

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