

HYPOTHESIS / AIMS OF STUDY

It is estimated that 50-80% of diabetics suffer from lower urinary tract (LUT) complications, with the commonest being diabetic bladder dysfunction (DBD, also known as diabetic cystopathy). Current treatments for DBD have both limited efficacy and pronounced side-effect profiles that limit patient adherence. Overall there is a real need to identify novel targets and strategies for treating DBD.

Because of the importance of MaxiK channel activity (a Ca²⁺- and voltage-gated potassium channel) in regulating bladder function, these channels have been intensely investigated as potential therapeutic targets for bladder pathology. At present, alterations in MaxiK-activity are believed to impact bladder physiology primarily through regulation of detrusor smooth muscle tone. However, the MaxiK channel is also expressed in non-contractile bladder mucosal tissue, where its function is poorly defined. We recently reported that inhibition of urothelial MaxiK results in changes in metabolism of both the sub-mucosa and detrusor [1]. Comparative analysis of the changes in metabolism caused by inhibition of urothelial MaxiK revealed that these are similar to changes in metabolism we have reported in the diabetic bladder [2]. Furthermore, we have shown that urothelial MaxiK plays a role in changes in the bladder physiology associated with diabetes.

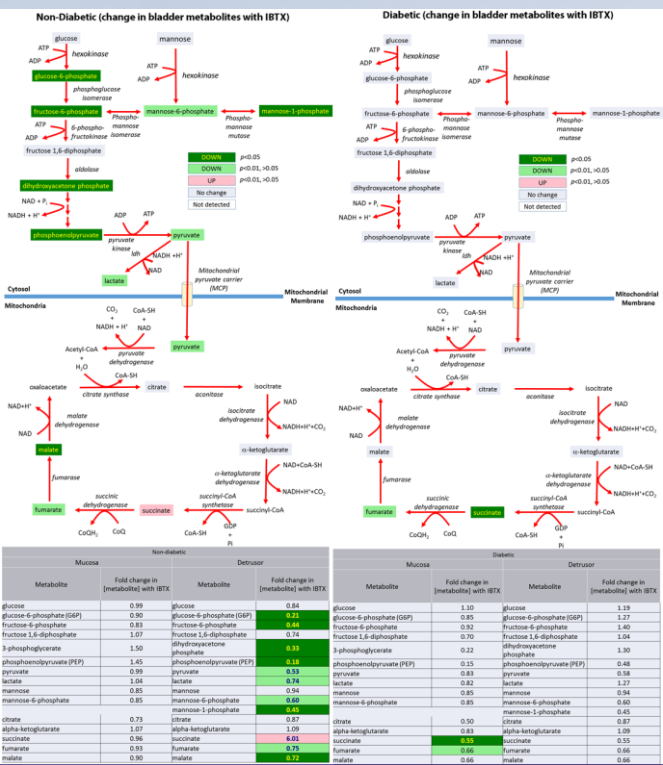
The aim of the present study was to support the hypothesis generated from observations, namely "Urothelial MaxiK regulates bladder metabolism; diabetes results in downregulation of urothelial MaxiK activity resulting in overall changes in bladder metabolism which are associated with the development of DBD".

METHODS

In this report we have compared metabolomics data on the bladder from our published studies that occur as a result of diabetes (in a rat model of streptozotocin-induced type 1 diabetes (T1D) [1] or through chemical inhibition, (instillation of iberiotoxin (IBTX)) into the bladder lumen) [2]. We have compared the expression of MaxiK between non-diabetic and diabetic rat detrusor and urothelial tissue by quantitative RT-PCR and Western Blot analysis. Using the yeast two-hybrid screen and co-immunoprecipitation (CoIP) we have screened for potential direct interaction between MaxiK and key enzymes of metabolism.

RESULTS

The change in the metabolomic profile caused by IBTX [1] in the non-diabetic animals was similar to the change in the metabolic profile of diabetic compared to non-diabetic detrusor [2]. Both diabetes and IBTX resulted in similar alterations of glycolysis, the TCA cycle and oxidative phosphorylation. Furthermore, when IBTX treatment was repeated in one-month STZ-diabetic animals, IBTX treatment no longer affected several metabolic profiles including glycolysis, the TCA cycle, polyunsaturated fatty acids, lysolipids, and sphingolipid.



By quantitative RT-PCR we demonstrated that MaxiK expression was significantly reduced at the transcriptional level in bladder from STZ diabetic rats (one-month) compared to non-diabetic controls. Western blot analysis demonstrated reduced expression of MaxiK protein in both mucosa and detrusor tissues from diabetic animals.

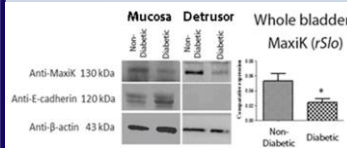


Figure 1: Left Panel: Western blot analysis demonstrating the expression of MaxiK channel is decreased at translational level with diabetes both in the bladder mucosa and detrusor. Right Panel: Transcription of the gene encoding the rat MaxiK (r/slo) in the bladder is decreased with diabetes (determined by quantitative RT-PCR). * = P value < 0.05 (Note: probing with E-cadherin was used to demonstrate the purity of mucosal preparation).

Using the yeast two-hybrid screen we identified a novel interaction between MaxiK and ADP-dependent glucokinase (ADPGK, EC 2.7.1.147). This enzyme catalyzes the ADP-dependent phosphorylation of glucose to glucose-6-phosphate and may therefore play a role in glycolysis. We have confirmed this interaction in co-transfected HEK293 cells with plasmids expressing the gene encoding human MaxiK (hsl0) tagged with cMYC (hsl0-cMYC) and ADPGK tagged with green fluorescent protein (ADPGK-GFP). Analysis of proteins co-immunoprecipitated with MaxiK by Mass-Spectroscopy identified another potential direct binding partner involved in glycolysis, glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ED 1.2.1.12) (approximately 3.5-fold more than ADPGK).

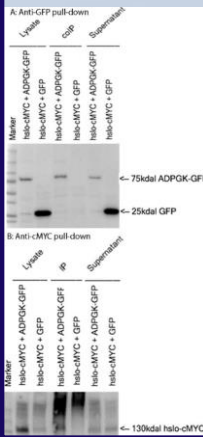


Figure 5 ADPGK was co-transfected into HEK293 cells with plasmids expressing hsl0-cMYC (human slo gene tagged with cMYC) and ADPGK-GFP (ADPGK tagged with GFP). Untransfected cell lines acted as a negative control. Protein was isolated from the cell-lines after approximately 12 hours and used for co-immunoprecipitation (CoIP) with the anti-cMYC antibody. An SDS-PAGE gel was run containing the protein lysates, cMYC-immunoprecipitated proteins and a molecular weight marker, followed by transfer of protein to a PVDF membrane. Western blotting was performed using anti-GFP as the primary antibody to detect ADPGK-GFP (upper panel) and then stripped and probed a second time using anti-anti-cMYC (lower panel) as the primary antibody to detect hsl0-cMYC.

Table 2. Results of Mass spectrometry of MaxiK interacting proteins

Identified proteins	Accession Number	Relative abundance
Glyceraldehyde-3-phosphate dehydrogenase	sp P04406 G3P_HUMAN	53
Pyruvate kinase PKM	sp P14618 PKYM_HUMAN	43
Fructose-bisphosphate aldolase A	sp P04075 ALDOA_HUMAN	37
ATP-dependent 6-phosphofructokinase	sp Q01813 PFKAF_HUMAN	36
D-3-phosphoglycerate dehydrogenase	sp Q43175 SERAH_HUMAN	16
Phosphoglycerate mutase 1	sp P18669 PGAM1_HUMAN	14
ADP-dependent glucokinase	sp Q288R6 ADPGK_HUMAN	16
Glucose-6-phosphate isomerase	sp P06744 GPI_HUMAN	7
Hexokinase-2	sp P52789 HK2C_HUMAN	8
Glucose-6-phosphate 1-dehydrogenase	sp P11413 G6PD_HUMAN	2

DISCUSSION

We demonstrate that inhibition of urothelial MaxiK by IBTX causes similar changes in overall bladder metabolism as seen with diabetes. A possible explanation for this observation comes from the observation of reduced expression of MaxiK at both the transcriptional and translational level in the diabetic bladder; the effect of lowered levels of MaxiK expression that occurs with diabetes would be equivalent to reduced activity resulting from the chemically induced inhibition of MaxiK. We present evidence of novel interactions between MaxiK and key enzymes of metabolism. The reduced levels of MaxiK expression observed with diabetes could perturb these interactions, suggesting a mechanism by which diabetes effects metabolism. If direct interaction with key enzymes is indeed the mechanism by which MaxiK regulates metabolism, this would support a gene therapy strategy to treat to DBD where MaxiK is overexpressed; however, pharmaceutical approaches aimed only to increase MaxiK activity would not be supported.

CONCLUSION

Overall our data provides evidence that urothelial MaxiK can modulate overall bladder metabolism, potentially through direct interaction with key enzymes of energy metabolism. The down-regulation of bladder MaxiK associated with diabetes could potentially perturb the activity of these enzymes and thereby suggest a mechanism for the effect on metabolism.

REFERENCES

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