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METABOLOMIC AND EPIGENOMIC STUDIES PROVIDE EVIDENCE FOR "HYPERGLYCEMIC MEMORY" IN DIABETIC BLADDER DISEASE.

Hypothesis / aims of study

Approximately 50-80% of diabetic patients suffer from lower urinary tract complications, the most common of which is diabetic bladder dysfunction (DBD). The clinical symptoms of DBD include storage problems (overactive bladder and urge incontinence) and voiding problems (poor emptying and overflow incontinence). At present, treatments for DBD target hyperglycemia and/or the clinical symptoms. However, data (from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study in T1D) suggest that after a certain time of hyperglycemia, the progression of diabetic complications cannot be reversed even if normal glucose levels are achieved. The failure of glycemic control to reverse pathologies associated with diabetes is called "hyperglycemic memory" and is well-documented for certain complications of diabetes, such as in retinopathy, nephropathy and macrovascular complications- but there are few (if any) reports of this phenomenon in DBD. The cause of "hyperglycemic memory" is attributed to epigenomic modification resulting from hyperglycemic-mediated changes in metabolism which modify genomic DNA .

Diabetes can be considered a "metabolic disease" in that the pathophysiology's associated with diabetes result from deleterious changes in metabolism. Therefore, we have used metabolomics to provide evidence of "hyperglycemic memory" in the bladder by looking for metabolic pathways that are not reversed after glycemic control by insulin, and used epigenomics to demonstrate that in genomic loci containing the genes involved in these pathways, epigenetic modifications are also not reversed by insulin treatment.

Study design, materials and methods

All experimental protocols were approved by our Institutional Animal Care and Use Committee. Five male F344 rats were made diabetic for 4-months using streptoztocin treatment (STZ- a model of Type 1 diabetes). A second group of five male F344 rats were made diabetic for 3-months followed by 1-month of intensive insulin treatment. A control group consisted of five non-diabetic, age matched controls. At the end of the study animals were euthanized and bladder urothelium and detrusor tissue isolated. Metabolomic profiling was performed on each tissue by Metabolon Corp. ANOVA was used to identify significant changes in the expression of metabolites. In addition each tissue was assayed for total levels of DNA methylation and genome wide DNA-methylation profiling through use of a HELP-tagging assay.

Results

Metabolomic analysis demonstrated that diabetes caused major changes in the energy generating pathways of both detrusor and urothelium, resulting in increased oxidative stress and generation of AGE products. AGE products have been implicated in causing epigenetic modifications. In addition, diabetes caused reduced metabolite levels of methionine and betaine, which would result in a "methyl-donor substrate" deficiency, potentially leading to decreased methylation of genomic DNA. Although glycemic control through the use of insulin did appear to reverse the majority of the effects of diabetes on the energy generating pathways in both detrusor and urothelium, in urothelium metabolites involved in the biosynthesis of lipid membrane components (dihomo-linolenate and arachidonate) did not respond well to treatment. This potentially could affect signalling pathways between the urothelium and detrusor, and thereby bladder physiology.

We demonstrated that diabetes resulted in epigenetic modification; the percentage of total methylated genomicDNA (5-mC) was approximately 13% less in diabetic compared to non-diabetic controls. In diabetic animals with 1-month of insulin treatment there was no significant reversal of methylated genomicDNA. The use of genome wide DNA-methylation profiling allowed us to focus on epigenetic modification of specific loci. For example, we analysed the methylation patterns at the genomic loci encoding enzymes (phospholipase B1, P1b1 and acyl-CoA thioesterase2, Acot2) responsible for the synthesis of the metabolites that were not reversed by insulin treatment (arachidonate and dihomo-linolenate, respectively). The methylation pattern at the two loci encoding these genes differ between the diabetic and non-diabetic bladder; however insulin treatment of diabetic animals had little effect on the methylation pattern. This is in contrast to the loci encoding the enzymes responsible for metabolites involved in energy generating pathways which have levels normalized by insulin treatment; the methylation patterns after insulin treatment more closely resemble that of the non-diabetic genome.

Interpretation of results

This is the first report providing evidence of "hyperglycemic memory" as a factor in poor treatment outcomes for glycemic control for DBD. We demonstrate that not all of the metabolic changes in the bladder resulting from diabetes are reversed by glycemic control though insulin. For example, metabolites involved in the synthesis of lipid membrane components (dihomo-linolenate and arachidonate), which have the potential to disturb urothelial-detrusor signalling pathways and thereby overall bladder physiology, did not respond well to insulin treatment. Furthermore, genome wide DNA-methylation profiling demonstrated that methylation patterns at the genomic loci encoding enzymes responsible for their biosynthesis were not reversed by insulin treatment, thereby providing evidence of epigenetic factors in bladder "hyperglycemic memory".

Concluding message

These studies are the first to provide evidence that epigenetic factors play a role in "hyperglycemic memory' associated with DBD.

Disclosures

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