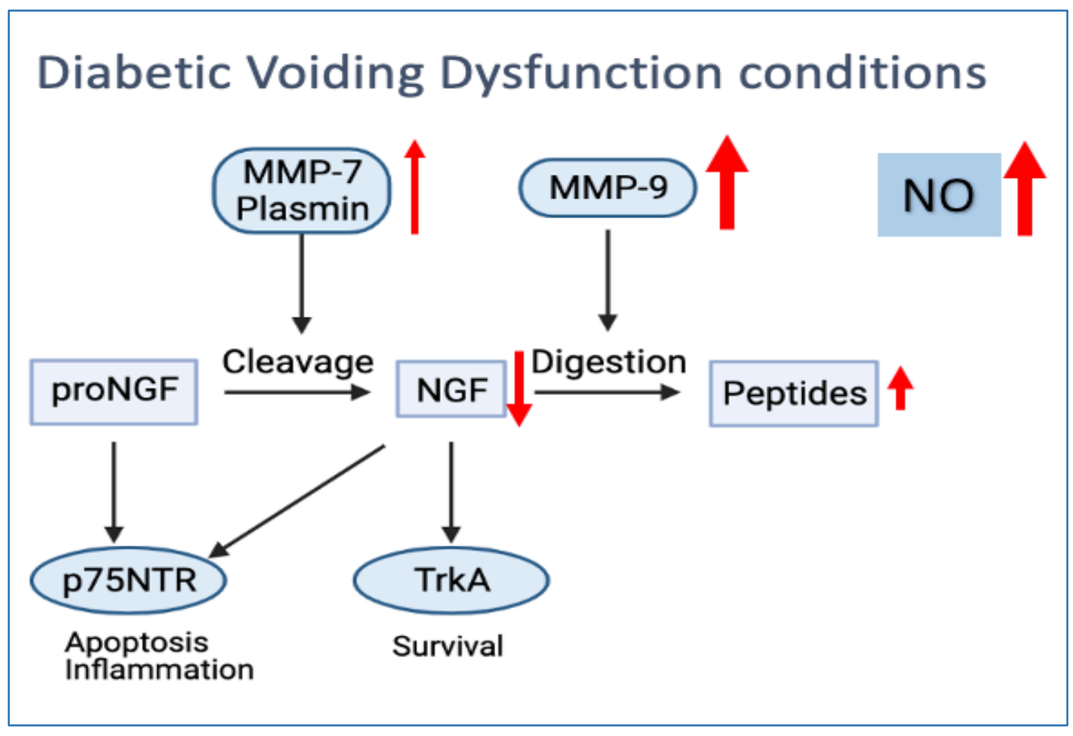
The role of Nitric oxide in the secretion of NGF in bladder cells in culture

Background

- **NGF**: Regulation of growth, survival and differentiation of neurons when bound to TrkA receptor
- **proNGF**: Precursor to NGF; inflammation and cell death when bound to $p75^{NTR}$
- **MMP-7** and **plasmin**: Cleavage of proNGF to NGF
- **MMP-9**: Digestion of NGF to peptides
- **NGF/proNGF ratio establishes a balance between cell survival vs inflammatory/apoptotic processes**
- **Urine samples of female patients with diabetic voiding dysfunction (DVD) are characterized by low levels of nerve growth factor (NGF) and elevated concentrations of nitric oxide (NO).**



Objective: Determine how a **high glucose environment** affects synthesis of **nitric oxide** and how this influences the secretion of **NGF** and **proNGF** in bladder cells in culture

Methods

- Primary urothelial and smooth muscle cell cultures were isolated from rat bladders, grown *in-vitro.* Incubation with glucose (24 hours, 27mM and 55mM respectively), and SNP (24 hours, 300µM)
- NO levels measured by Greiss reaction
- Crispr-Cas9 knockdown of genomic MMP-9
- Enzymatic kits and immunblotting (MMPs)
- RT-qPCR (NGF mRNA levels)
- ELISA for protein quantification (cGMP, NGF, proNGF)
- Results presented as mean and standard error of the mean (SEM) for all values. Statistical significance was presented as a p value where $p<0.05$, $p<0.01$ and $p<0.001$

Results

Hyperglycemic medium decreases NGF secretion through generation of nitric oxide

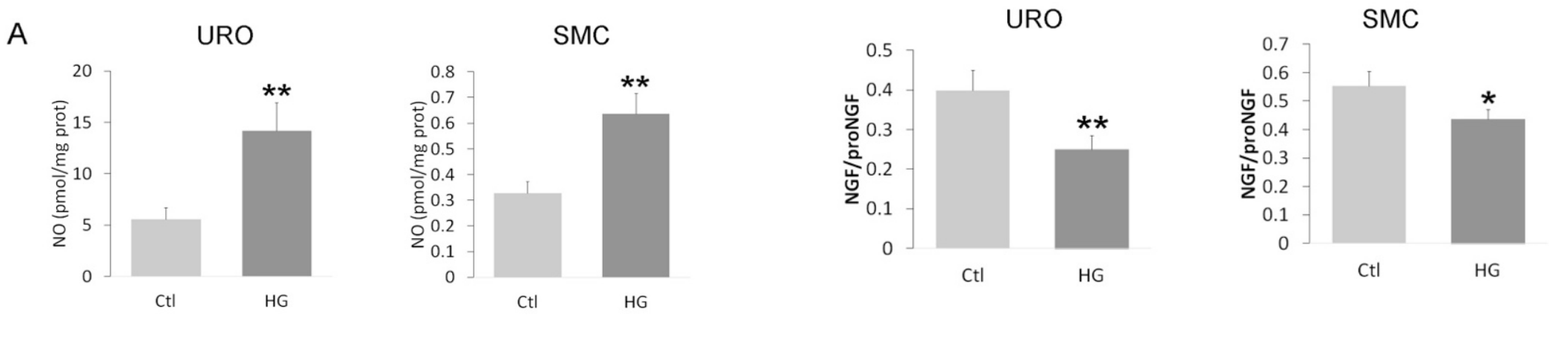


Fig. 1. A hyperglycemic medium (HG) increases nitric oxide (NO) secretions in both urothelial (URO) and smooth muscle cells (SMC). Hyperglycemic conditions also lead to decreased NGF secretion, stable proNGF levels and a decrease in NGF-to-proNGF ratio in both cell types

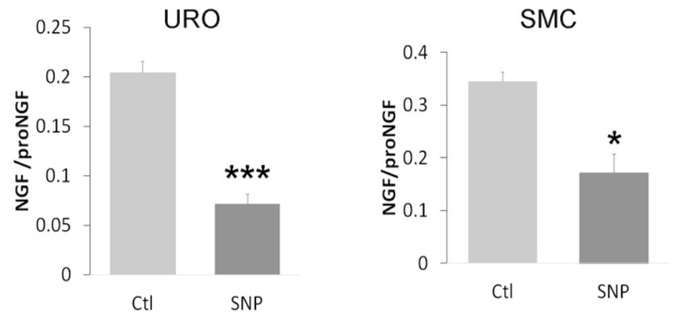


Fig. 2. In order to get rid of other cellular effects potentially linked to hyperglycemia, UROs and SMCs were incubated for 24-hours with the NO generator SNP (300 µM). **I**ncubation led to **decreased NGF** secretion and **NGF/proNGF** in UROs and SMCs, mimicking hyperglycemia

cGMP mediates the effect of nitric oxide on decreased NGF secretion

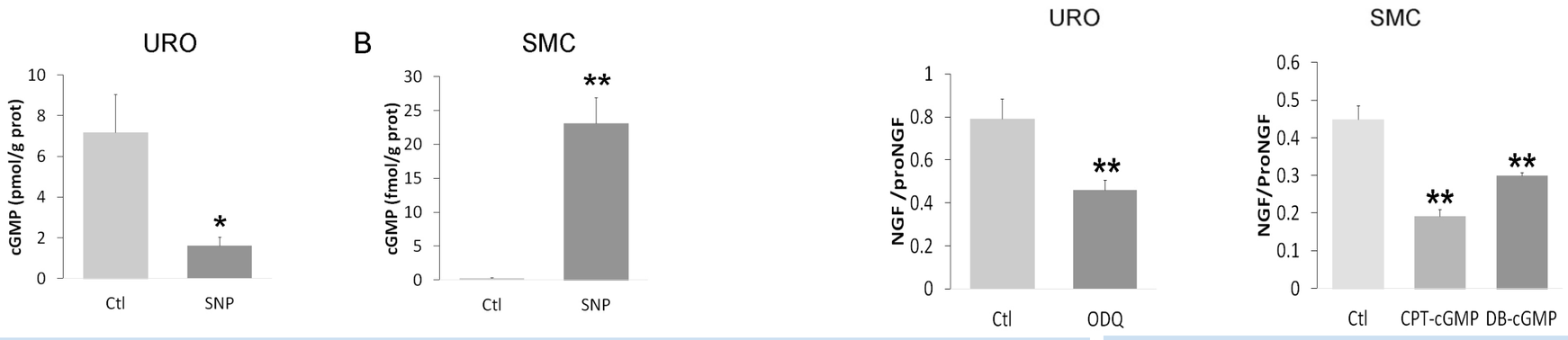


Fig 3. Cyclic GMP mediated NO metabolism pathways and displayed a different pattern between UROs and SMCs incubated with SNP. . The cGMP synthetase inhibitor **ODQ** and **cGMP analogs** mimicked these effects.

MMP-9 is responsible for NGF decrease in urothelial but not smooth muscle cells

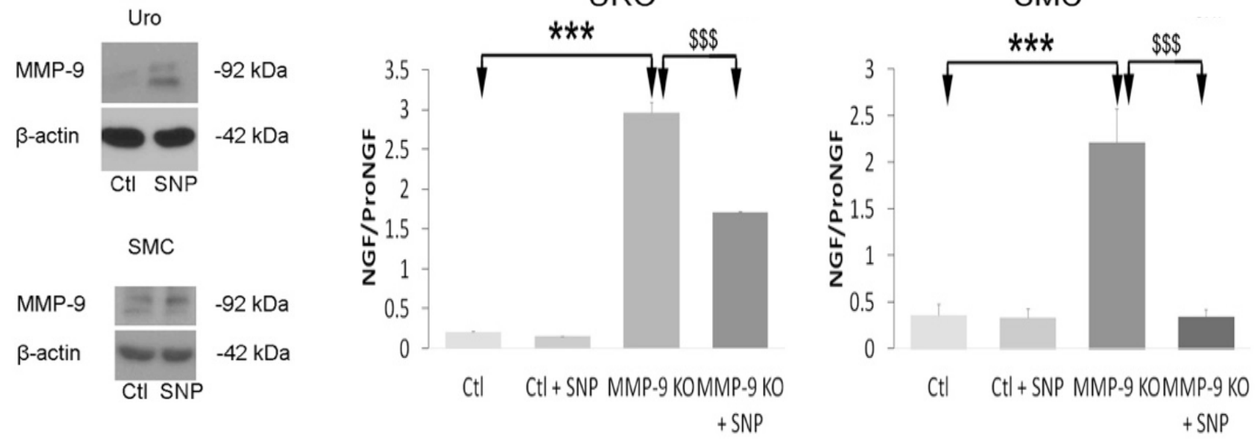


Fig. 4. Immunoblotting revealed increased MMP-9 protein content in UROs only.

Deletion of MMP-9 genomic sequence using Crispr-cas9 in UROs revealed that the effect of SNP on NGF is partially dependent of MMP-9

In SMCs the effect of SNP is independent of MMP-9

SNP inhibits MMP-7 decreasing conversion of proNGF to NGF in smooth muscle cells only

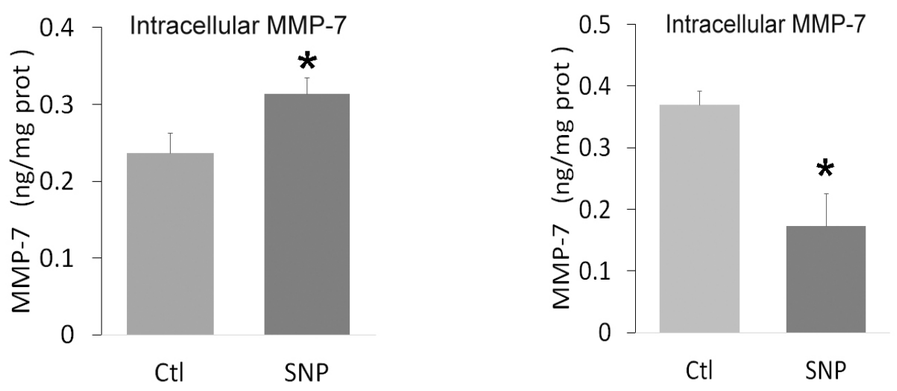


Fig. 5. MMP-7 activity increased in UROs treated with SNP, suggesting a compensatory mechanism

Decrease in MMP-7 can explain the decrease of NGF in SMCs

Conclusions :

- High glucose increased NO release, leading to decrease in NGF and NGF-to-proNGF ratio
- In **UROs**, NGF secretion is controlled by NO through decreased in cGMP signaling and increase in MMP-9 activity
- In **SMCs**, decreasing MMP-7 activity contributes to lower NGF secretion through NO and increased cGMP
- These data confirmed our clinical observations and suggest that increased glycemia through NO could be part of the pathological process leading to OAB