The GPR91 receptor is activated in the urothelium and involved in detrusor contractility

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BACKGROUND

Metabolic syndrome is associated with **overactive bladder (OAB)** and increased circulating levels of **succinate**.

Succinate was recently identified as a major metabolic switch activating pathological pathways in some body organs through its receptor GPR91 (SUCNR1).

Our previous findings on urothelial and detrusor smooth muscle cell (SMC) cultures showed that succinate acts on GPR91 receptor on urothelial cells to activate phosphorylation of the MAPK kinase pathway (ERK and JNK), increase calcium mobilization, release of nitric oxide (NO), reduce basal PGE2 secretion and prevent cAMP accumulation.

Succinate may interfere with the physiological inhibitory effect of urothelium via GPR91 receptor and can be involved in the development of OAB in the context of metabolic syndrome.

Aim: The aim of this study was to identify the consequence of an absent GPR91 receptor on the urinary bladder function at the cellular and organ levels.

METHOD

Urothelial cells Knockdown

- **Urothelial cells (UT)** from Sprague Dawley (SD) rat bladders were cultured and characterized.
- Knocked down (KD) urothelial cell line was established by retroviruses containing **GPR91 shRNA** and the KD of the gene was confirmed by RT-PCR.
- After confluency, cells were **incubated with succinate** [200 µM] and **Western Blotting, ELISA or other enzymatic essays** were used for cytokines estimation.

GPR91 KO mice

- For the ***in vivo*** experiments, ***GPR91*** **Knockout (KO)** mice were purchased and KO was confirmed by genotyping.
- After surgical implantation of bladder catheter, **awake cystometry** was conducted to compare the baseline parameters of the KO mice to control C57BL6 mice.
- **Organ bath studies** on fresh bladder strips (with or without urothelium) were conducted. Bladder strips were stimulated with **KCl, carbachol and electrical field** then succinate was added to study the acute effect of succinate on normal and KO bladders.

RESULTS:

UT KD

- The KD of GPR91 in the urothelial cells prevented all responses of succinate stimulation detected in normal cells;
- That is, phosphorylation of ERK and JNK was annihilated in the KD cells, calcium mobilization was not increased on exposing KD to succinate compared to their response to ATP, release of nitric oxide elicited by succinate was completely prevented and the effect on cAMP secretion was severely impaired. (figure 1-B to E).

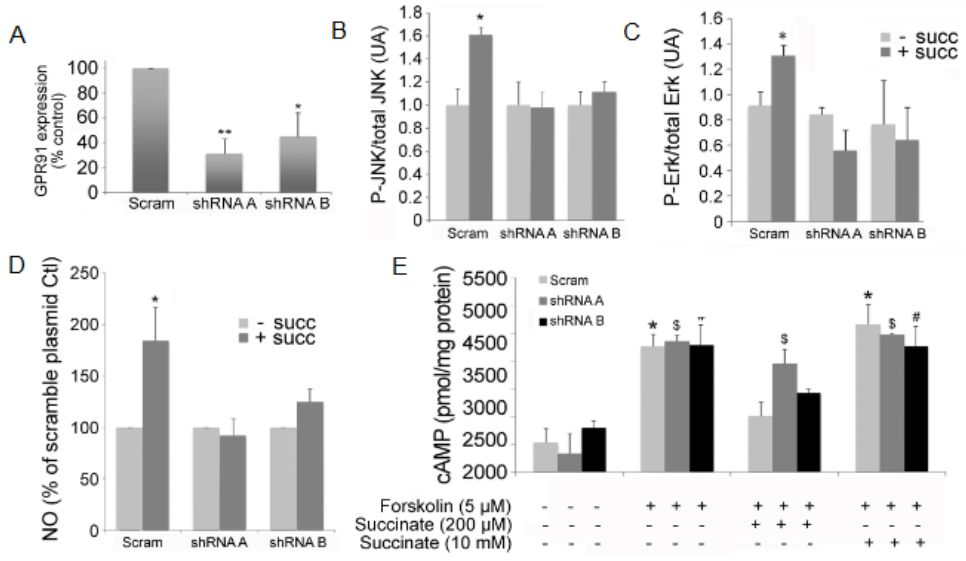

Figure 1: GPR91 shRNA knockdown urothelial cells A) GPR91 expression in scrambeled, A and B shRNA constructs. *p<0.05, **p<0.01 B, C and D) JNK and ERK phosphorylation was significantly reduced in KD UT, nitric oxide (NO) production in the medium was abolished in response to incubation with [200µM] succinate. E) The inhibitory effect of succinate at [200µM]on cAMP by forskolin was reduced in KD UT constructs. one-way ANOVA, *P<0.01 compared with Scram control, $P<0.01 compared with shRNA A control, #P<0.01 compared with ShRNA B control. (n=6)

CONCLUSION 1

The role of succinate in the activation of the G-protein coupled receptor GPR91 through Gi and Gq pathways in UT is confirmed.

The resulting change in the released mediators can interfere with the normal physiological inhibitory effect of the urothelium on the suburothelial sensory tissue.

GPR91 KO MICE

- **Cystometry** data on the animal models showed **comparable urodynamic parameters** in the two groups despite the tendency of the KO mice to have **higher glycaemic index** and **heavier bladders**.
- **Organ bath studies** showed that the baseline response to KCl and carbachol are not significantly different in the strips between groups. Contraction to electrical field stimulation in KO strips was stronger especially when the urothelium is intact. (figure 2).

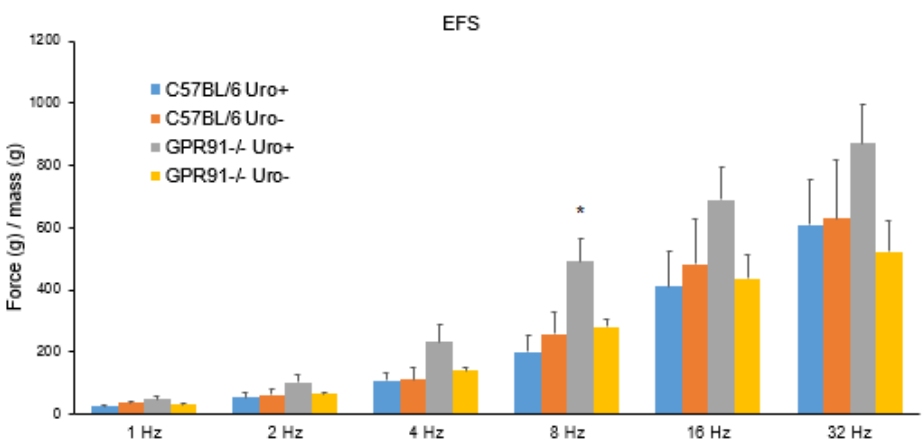

Figure 2: Electrical field stimulation response in the organ bath experiment. KO bladder strips with urothelium showed stronger response. (*p<0.05, two way-ANOVA)

CONCLUSION 2

The urothelium in the KO animals plays a more important role in neuronal mediated detrusor contraction based on our organ bath findings, suggesting that the receptor absence makes the detrusor more neuronally-dependent on urothelium for contraction.

BOTTOM LINE

Succinate effect through the GPR91 receptor in urothelial cells provides another potential pathophysiologic mechanism involved in the development of OAB in the context of metabolic syndrome.

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