651
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THE GPR91 RECEPTOR IS ACTIVATED IN THE UROTHELIUM AND INVOLVED IN DETRUSOR CONTRACTILITY

Hypothesis / aims of study
The aim of this study is to identify the consequence of an absent GPR91 receptor on the urinary bladder function at the cellular and organ levels. 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 second messenger (ERK and JNK), increase calcium mobilization, release of nitric oxide (NO), reduce basal PGE2 secretion and prevent cAMP accumulation. Succinate as a metabolite increased in metabolic stress conditions (1) 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 (2). To confirm that the activated signalling pathways were succinate/GPR91-related, we have reproduced the results on GPR91 knockdown (KD) urothelial cell line and GPR91 Knockout (KO) mouse line.

Study design, materials and methods
Urothelial and detrusor smooth muscle cells from Sprague Dawley (SD) rat’ bladders were cultured and characterized. Urothelial cells were infected by retroviruses containing either a scrambled, or GPR91 shRNA construct A or B and the KD of the gene was confirmed by RT-PCR (figure 1-A). After confluence, cells were incubated with succinate [200 μM] and cell lysates or culture medium were used to estimate released cytokines or secretions by Western Blotting, ELISA or other enzymatic essays. For the in vivo experiments, GPR91 KO mice were purchased from external source 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 age- and weight-matched C57BL6 mice. Fresh bladders were extracted and cut into strips which were either stripped from urothelial cells or not and used for organ bath studies. Bladder strips were stimulated with KCl, carbachol and electrical field then succinate was added to study the acute effect of succinate on normal (KO) bladders.

Results
The KD of GPR91 in the urothelial cells prevented all responses of succinate stimulation detected in the wild type cells. That is, phosphorylation of ERK and JNK was significantly lower in the KD cells, calcium mobilization was not increased on exposing KD succinate to compare their response to ATP, release of nitric oxide (NO) was significantly lost and the inhibitory effect on cAMP secretion was not seen at 200 μM concentration (figure 1-B to E). Cystometry data on the animal models showed comparable urodynamic parameters in the two groups except for 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).

Interpretation of results
The results confirm the role of succinate in the activation of the G-protein coupled receptor GPR91 through Gq and Gi pathways (3) in the urothelial cells. The resulting change in the released mediators can interfere with the normal physiological inhibitory effect of the urothelium on the suburothelial sensory tissue. Under normal physiological conditions, GPR91 KO animals did not show different urodynamic parameters. 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.

Concluding message
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. Further work is underway to understand the succinate/GPR91 effect on other cellular components of the bladder which, when taken together, would contribute to a better understanding of cellular interaction and potential development of effective therapeutic targets.

References

Disclosures
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Species: mouse
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Figure 1: **GPR91 shRNA knockdown urothelial cells** A) GPR91 expression in scrambled, A and B shRNA constructs. *p<0.05, **p<0.01 B, C and D) JNK and ERK phosphorylation was significantly reduced in KD urothelial cells, 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 urothelial cell 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)

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)