

## UROTHELIAL CELLS EXPRESS A FUNCTIONAL SUCCINATE RECEPTOR GPR91

### Hypothesis / aims of study

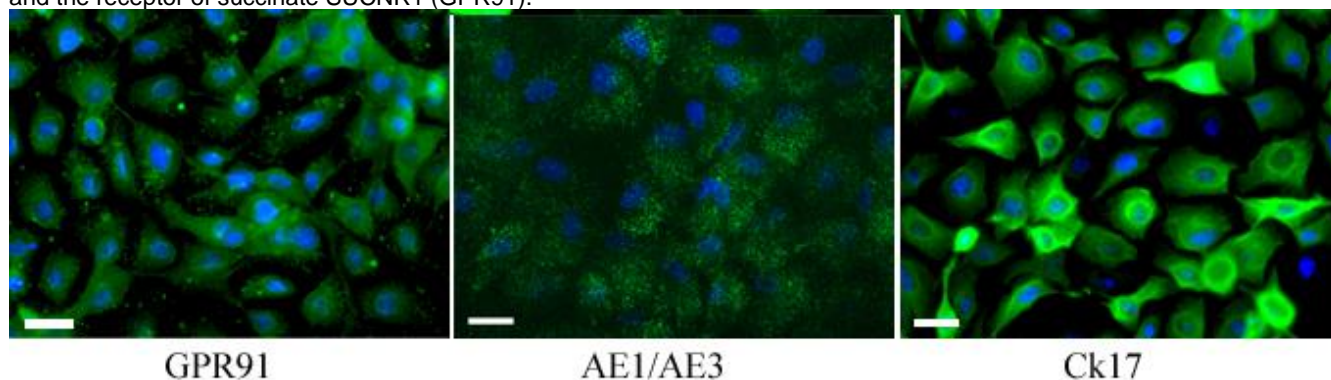
Lower urinary tract symptoms are associated with the metabolic syndrome. Increased succinate production is detected in the presence of hyperglycemia and hypoxemia, as with diabetes mellitus and metabolic syndrome, which is strongly associated with overactive bladder syndrome. Succinate was recently identified as a major metabolic switch controlling metabolic functions in the body through its receptor GPR91 (SUCNR1). The aim of our study is to determine how succinate modulated bladder contractility.

### Study design, materials and methods

Urothelial cells were isolated from Sprague-Dawley rat bladder using a collagenase IV method and grown in collagen IV-coated petri dishes. After confluency, cells were exposed to succinate then assessed by microscopy and immunoblotting analysis. Cyclic AMP was measured using an Elisa kit from Cayman Chemical Company.

### Results

Immunohistochemistry revealed that confluent cells express Cytokeratin 17, cytokeratins recognized by the AE1/AE3 antibody and the receptor of succinate SUCNR1 (GPR91).



Immunoblotting on urothelial cell extracts confirmed expression of GPR91. Incubation of cells with succinate (10<sup>-2</sup> M) results in phosphorylation of Erk and c-Jun amino-terminal kinases (JNKs) JNK, with no effect on the levels of Akt-308P, Akt 473P, iNOS, enos-1177P or enos-405P.

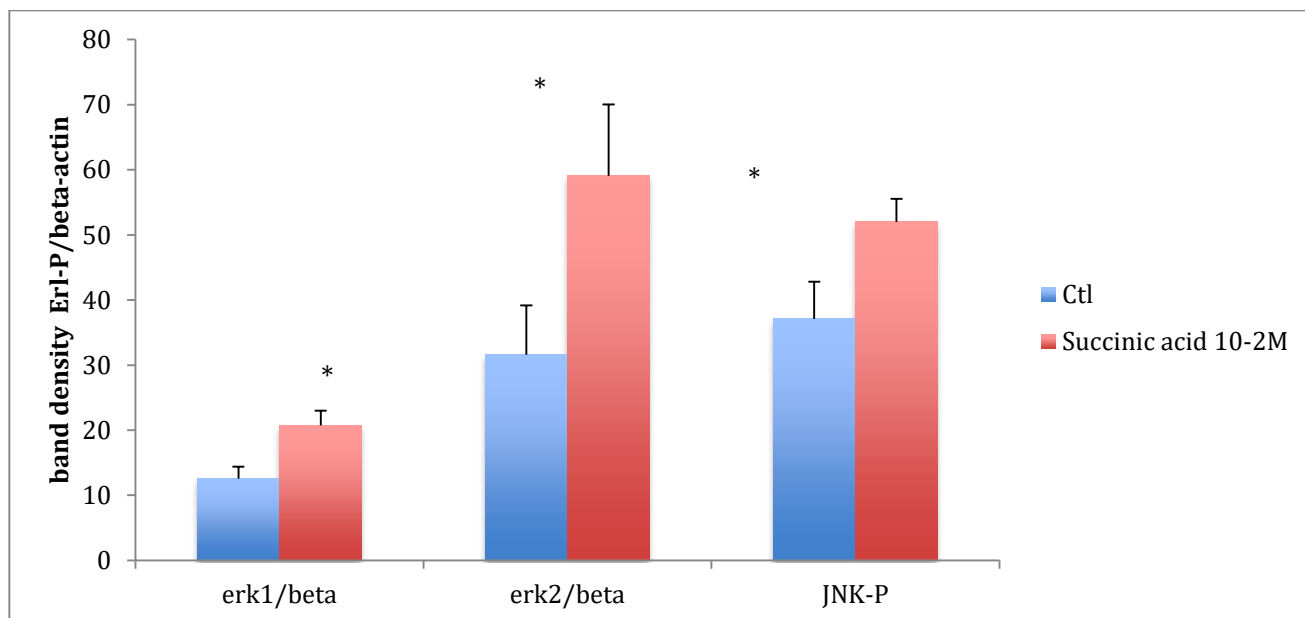


Figure 1. Urothelial cells were incubated in the presence or absence of succinic acid 10 mM and proteins were extracted to assess levels of Erk-P and JNK-P. (n=5) (student t-test: \*P<0.05)

Erk and JNK phosphorylation was not observed after exposure to alpha-keto glutarate or citrate (10 mM), two other intermediates of the citric acid cycle with no affinity for GPR91 receptor, while maleic acid, another GPR91 ligand, did. On the other hand, inhibition of phospholipase C by U73122 (5 microM) or inhibition of the MAPK pathway by PD98059 (10 microM), both completely inhibited increases of Erk-P elicited by succinic acid.

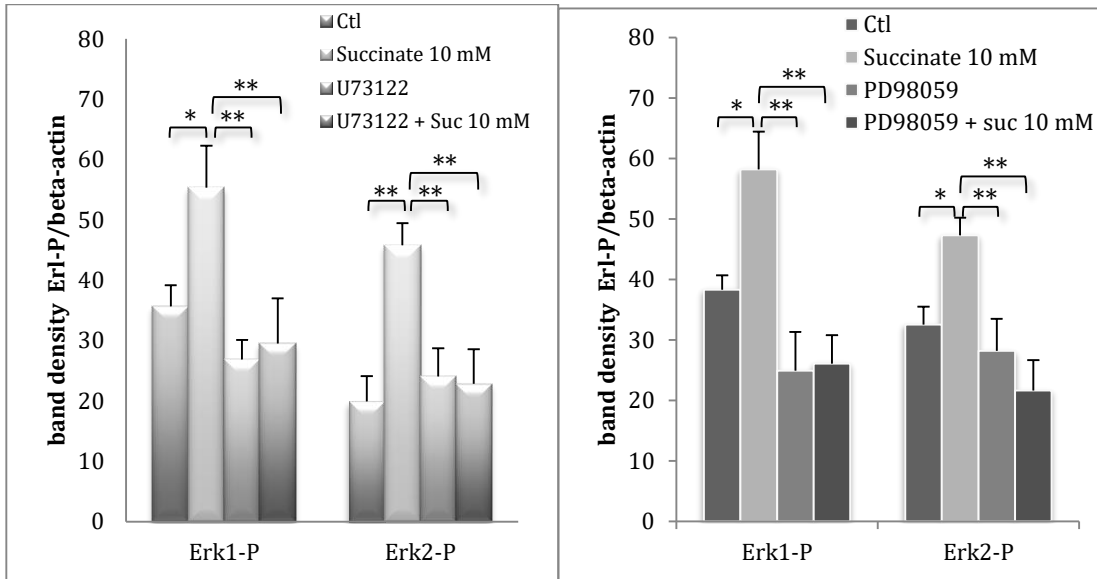


Figure 2. Urothelial cells were pre-incubated in the presence or absence of U73122 (inhibitor of Phospholipase C) or PD98059 (inhibitor of the MAPK pathway). Then succinic acid 10 mM was added to stimulate phosphorylation of Erk. (n=6) (Anova: \*P<0.05, \*\*P<0.01)

Finally, pre-incubation of cells with succinate dose-dependently decreased the concentrations of intracellular cyclic AMP stimulated by forskolin.

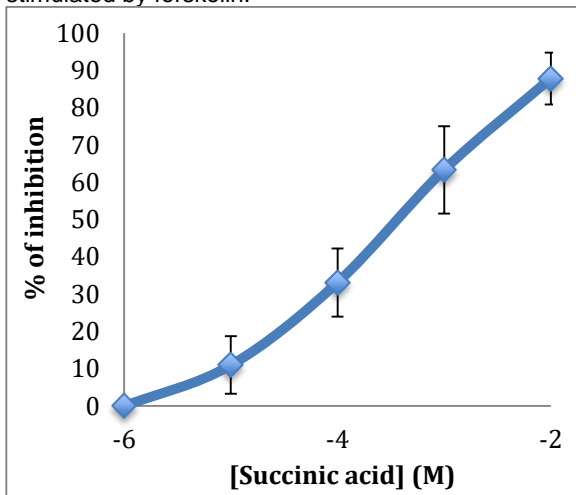


Figure 3. Cyclic AMP synthesis was stimulated in the presence of forskolin (10 microM). Increasing concentration of succinic acid then inhibited its synthesis. Results were expressed in percentage of inhibition of cAMP synthesis in the presence of forskolin alone (n=6).

#### Interpretation of results

GPR91 is expressed in urothelial cells. Binding of succinate triggers phosphorylation of Erk and JNK, a process that requires Phospholipase C and the MAPK pathway. On the other hand, GPR91 inhibits cyclic AMP production, suggesting the receptor is bound to protein Gi.

#### Concluding message

These results suggest that succinate controls major signalling pathways in urothelial cells by binding to its receptor GPR91. As succinic acid is linked to hypoxia and metabolic disease, understanding its effect on urothelial cells may clarify an underlying pathophysiology of overactive bladder.

#### Disclosures

#### Funding: FRQS

Diabete Quebec **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Sprague-Dawley Rats **Ethics Committee:** Animal Compliance Office McGill University