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MIRABEGRON AND SUCCINATE IN THE CONTROL OF BLADDER CONTRACTION

Hypothesis/aims of study: Mirabegron is a β3 adrenergic agonist used in the treatment of bladder overactivity [1]. Succinate, an intermediate of the Krebs cycle, is increased in inflammatory states and metabolic diseases [2,3]. We previously demonstrated that succinate controls urothelial cell intracellular pathways and secretion. The aim of this study is to determine if mirabegron efficiency might be affected in patients with overactive bladder with metabolic syndrome.

Study design, materials and methods: RT-PCR and qPCR were carried out on RNA from rat urothelial and smooth muscle cells (SMC) culture. Levels of cellular P-Erk and iNOS were evaluated by immunoblotting. Immunohistochemistry was performed on bladder sections from Sprague-Dawley rats. Levels of cyclic AMP and PGE2 were measured by Elisa kits. Nitric oxide (NO) was quantified using a spectrophotometric method. Male SD rats were intraperitonealy injected with saline or succinate (50 mg/Kg) for 4 weeks and bladder strips were placed in organ bath to measure contraction/relaxation.

Results:

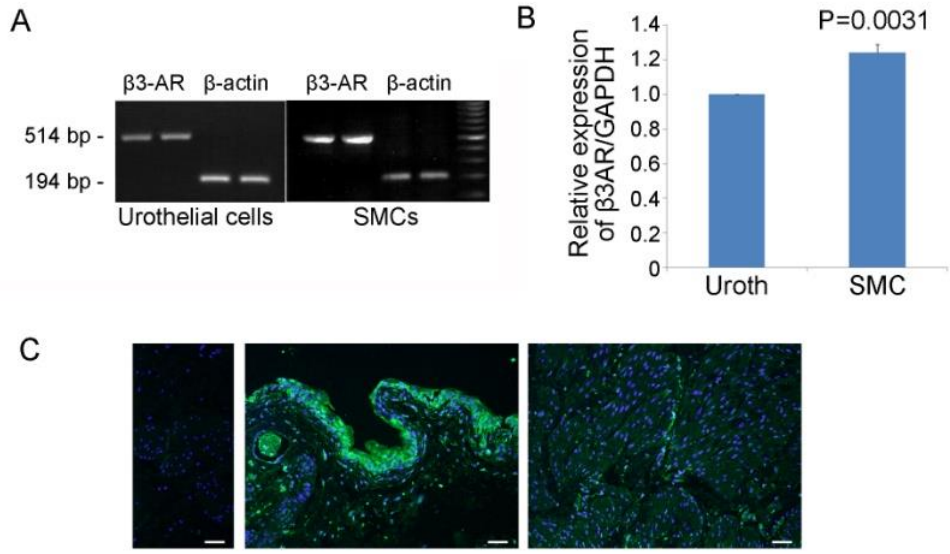

Figure 1. (A) Using RT-PCR, β3-adrenergic receptors were found in urothelial cell (left gel) and SMCs (right gel) in culture. β–actin was used as control. (B) Quantitative PCR revealed a slightly higher expression of β3-adrenergic receptors in SMC compared to urothelial cells (n=4). Student t-test P=0.0031. (C) Immunohistochemistry was used to reveal the localization of β3-adrenergic receptors in the bladder wall. From left to right, negative control without primary antibody, urothelium and connective tissue, detrusor muscle (bar = 100 µm).

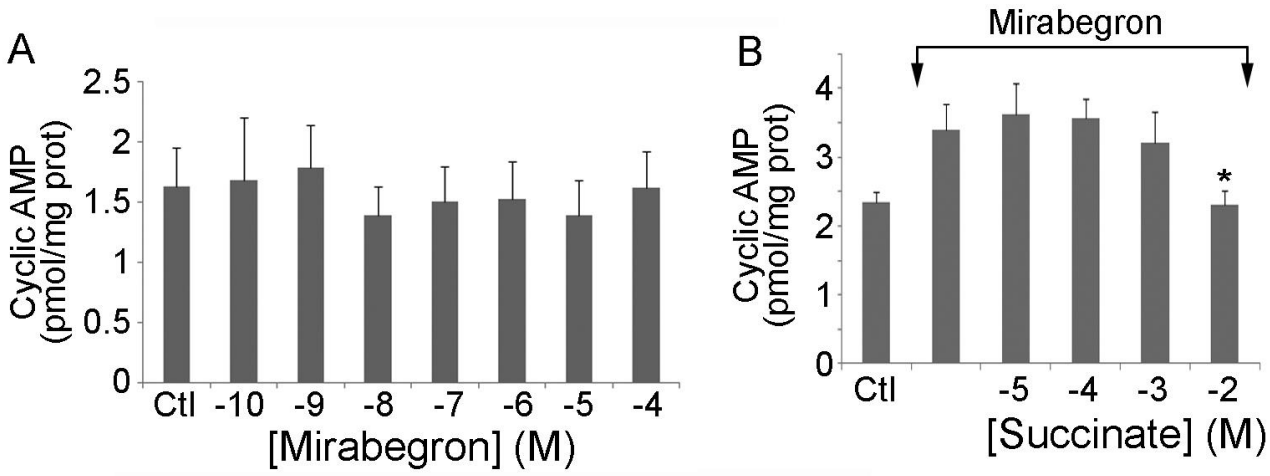

Figure 2. (A) Mirabegron (10^{-10} to 10^{-4} M) did not increase cyclic AMP levels in urothelial cells after 30 min of incubation (n=6). Similar results were obtained with 10 and 60 min of incubation (results not shown). (B) In SMCs, mirabegron (10 µM) incubated for 10 minutes increases cyclic AMP levels and were abolished only by high concentrations of succinate (n=6). (*P<0.05 compared to mirabegron alone)

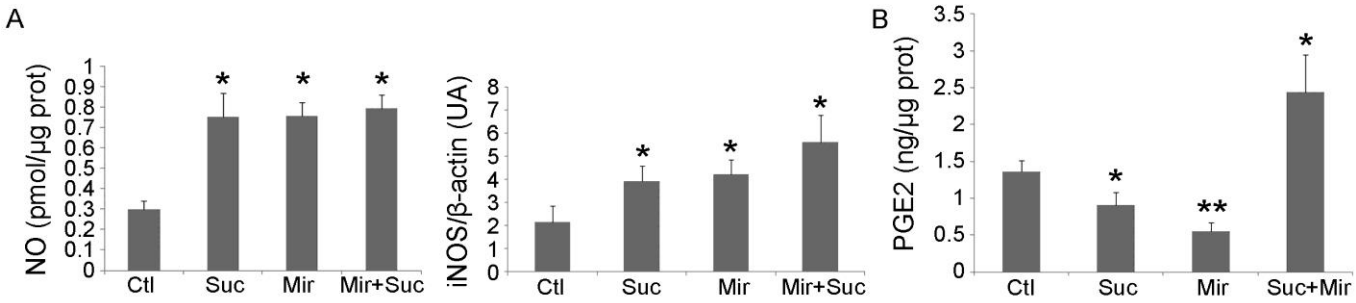

Figure 3. Succinate (200 µM) or Mirabegron (10 µM) were incubated with urothelial cells for 24 hours. (A) Both products increase secretion of nitric oxide (NO) and synthesis of iNOS with no synergistic effect (n=4). (B). Basal secretion of prostaglandin E2 (PGE2) was decreased by both compounds separately while their combination results in high release of PGE2. Similar experiments on SMCs revealed no change (results not shown). one-way ANOVA *P<0.05; **P<0.01 compared with control.

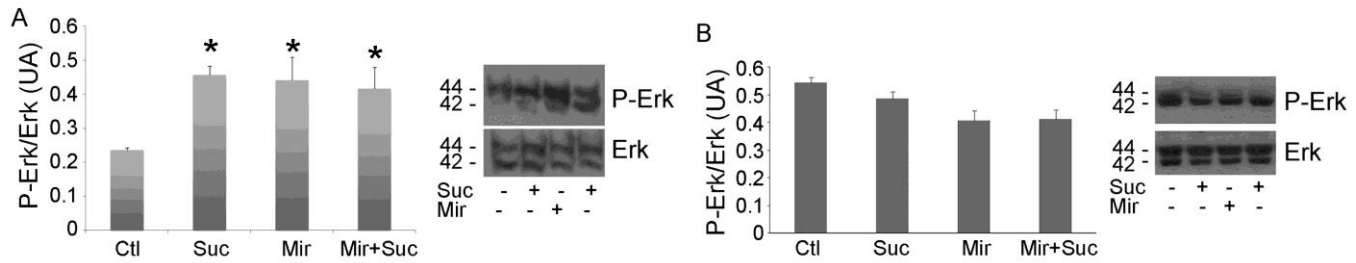

Figure 4. (A) Succinate (200 µM) or Mirabegron (10 µM) increases phosphorylation of second messenger JNK (not shown) and Erk in urothelial cells. Combination of both factors did not show synergy. (n=6) (B) In SMCs, neither succinate or mirabegron increase the amount of P-erk. Mirabegron alone stimulates phosphorylation of JNK with no additive effect of succinate (results not shown). (n=5). one-way ANOVA *P<0.05; compared with control.

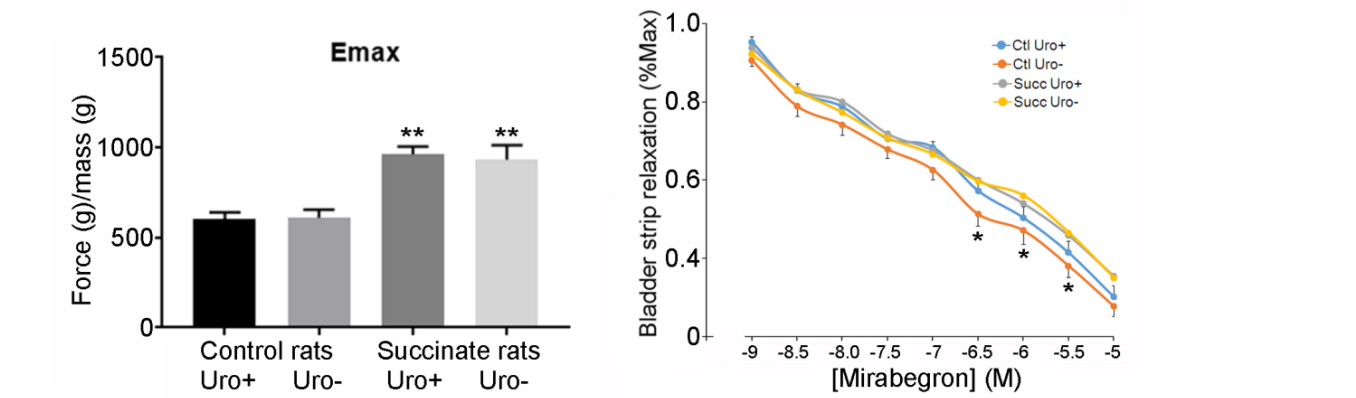

Figure 5. Chronic succinate increased EC50 of carbachol-elicited contraction, independently of the presence of urothelium (n=6, One-way ANOVA P<0.005, compared to respective control). Mirabegron relaxation was more efficient in control than succinate-treated rat strips without urothelium (n=6, ANOVA two-ways, *P<0.05).

Interpretation of results. We previously reported that succinate modulate urothelial cell activity with no effect on SMCs, and that this effect was mediated by GPR91 receptor. Similarly, mirabegron overlaps succinate properties on urothelial cells, including increase in P-Erk, in nitric oxide secretion and iNOS, among others, decrease in PGE2 secretion. Conversely, succinate counteracts cAMP increases by mirabegron in SMCs and increases PGE2 secretion when mirabegron is present. The latter effects are confirmed by organ bath findings, showing that in the absence of urothelium, strips without urothelium display the maximal mirabegron relaxation compared to the succinate-treated group. This may suggest that chronic succinate treatment impairs the response of the detrusor muscle to mirabegron.

Concluding message: Succinate is increased in inflammatory and metabolic diseases, including metabolic syndrome. Our results suggest that mirabegron may not provide optimal efficacy in a setting of elevated succinate, such as metabolic syndrome. These findings may lead to improved phenotypic characterization in patients with overactive bladder, in order to personalize treatment.

References
1. Imram et al. Mirabegron for overactive bladder: a novel first-in-class ß3agonist therapy. Urology J 10(3), 935-940.
2. Weihai et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature 429, 188-193
3. Mills E et al. Succinate: a metabolic signal in inflammation. Trends in Cell Biology 24(5), 313-320.

Disclosures
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