Mirabegron and succinate in the control of bladder contraction

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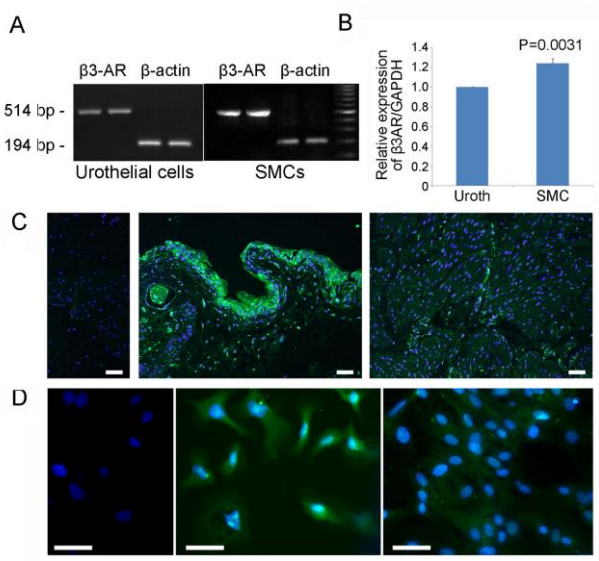
background

Mirabegron is a beta-3 adrenoceptor agonist prescribed for the treatment of overactive bladder disease, leading to muscle relaxation and increase in bladder capacity by binding to the receptors on smooth muscle cells.
Its action on urothelial cells has not been clearly established. Succinate, an intermediate of the Krebs cycle, has similar relaxing properties in vivo in rats and on bladder strips in organ bath.
The **aim of our study** is to determine the differential action of mirabegron on urothelial and smooth muscle cells, and if succinate could enhance mirabegron's relaxing effects.

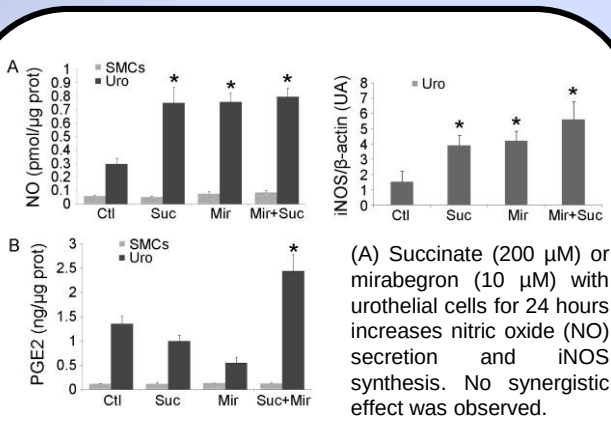
Materials

Urothelial and smooth muscle cells were isolated from female Sprague-Dawley rat bladder.
Cells were used at P2-P6 passages and processed for IHC, q and RT-PCR and immunoblotting.
Cyclic AMP and PGE2 were assessed by Elisa kits. Nitric oxide was measured using the Griess reaction. Organ bath was carried out on DMT equipment.

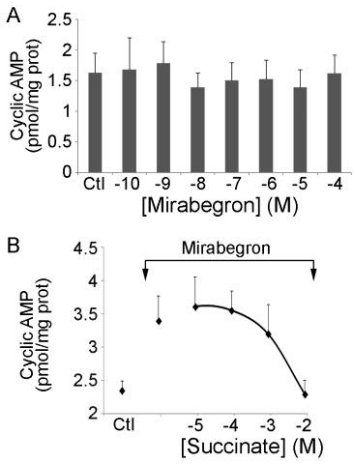
Results



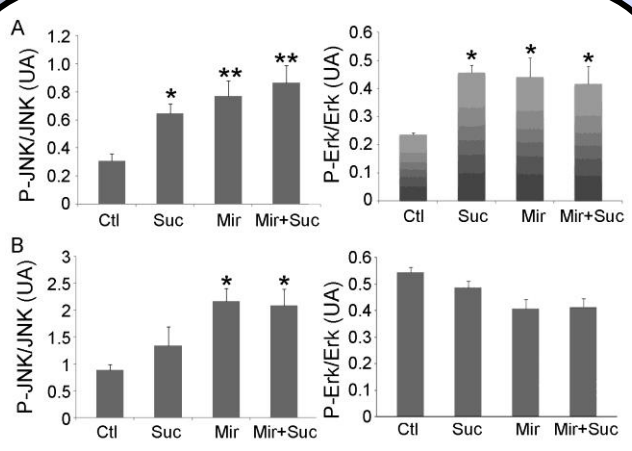
β3-adrenergic receptors are expressed in urothelial cells and SMCs in culture as revealed by PCR (A) and qPCR (B). (n=4) Student t-test P<0.005. (C) IHC was used to reveal the localization of β3-adrenergic receptors in the bladder wall (D)(From left to right, negative control, urothelium, detrusor muscle, bar = 100 µm) and in vitro (From left to right, negative control, urothelial cells, SMCs, bar = 50 µm).



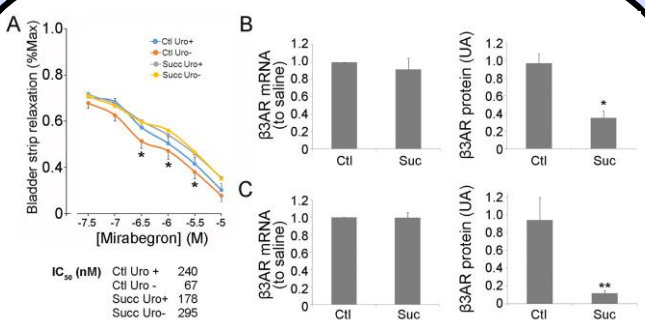
(A) Succinate (200 µM) or mirabegron (10 µM) with urothelial cells for 24 hours increases nitric oxide (NO) secretion and iNOS synthesis. No synergistic effect was observed. In SMCs, no effect were seen. (n=6).
(B) Succinate (10 mM) or mirabegron (10 µM) decreases basal secretion of prostaglandin E2 (PGE2) in urothelial cells whilecombination of both compounds results in high release of PGE2. No effect was seen on SMCs (n=6). One-way ANOVA *P<0.05 compared with control; **P<0.01 compared with control.



(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) increases cyclic AMP levels that were dose-dependently decreased by succinate (n=6). one-way ANOVA *P<0.05 compared with control.



(A) In urothelial cells, succinate (200 µM) or mirabegron (10 µM) increased phosphorylation of Jnk and Erk, with no additional effect when combined (n=5).
(B) In SMCs, mirabegron alone stimulated Jnk phosphorylation. No increase of P-Erk could be detected (n=5). ANOVA one-way, *P<0.05.



(A) SD rats were injected for 4 weeks with saline (Ctl) or succinate (Succ) (50 mg/Kg/day). Contraction of bladder strips was stimulated with carbachol (10^{-6} M) and recorded in an organ bath setting (DMT). Mirabegron inhibited contraction more efficiently in urothelium deprived strips but only in saline-treated rats (n=7). ANOVA one-way, **P<0.01.
(B) Bladder homogenates of succinate-treated rats displayed a decrease in the levels of β3-adrenoceptor protein compared to saline-injected controls, while their mRNA levels were unchanged (n=6).
(C) In SMCs incubated for 24 hours with succinate (200 µM), results were identical. Student t-test **P<0.01, *P<0.05

Conclusions

In urothelial cells, mirabegron and succinate both increase nitric oxide secretion, decrease PGE2 release and increases expression of survival genes. In smooth muscle cells, the effect of mirabegron is limited to increases in cyclic AMP while succinate has none. These results suggest that a combination of mirabegron and succinic acid could increase the direct relaxing effect of mirabegron on smooth muscle cells by enhancing the secretion of relaxing factors by urothelial cells.

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