

## BACKGROUND and AIMS

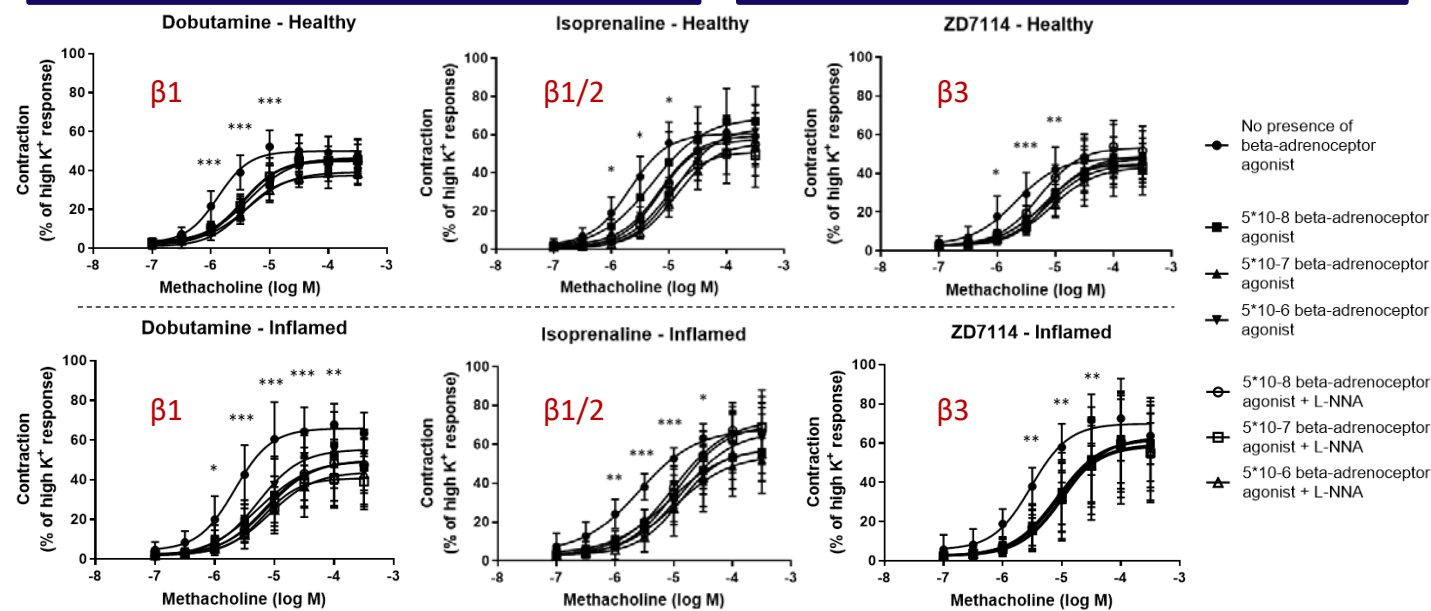
In patients suffering from overactive bladder (OAB) the detrusor is in a hypersensitive state. The exact nature of this hypersensitive state can vary, especially when comparing OAB patients with an otherwise healthy bladder to those with a concomitant inflammation. Mirabegron, a selective  $\beta_3$ -agonist, was recently approved for the treatment of OAB in combination with an antimuscarinic drug. Early reports on mirabegron suggested that part of the relaxatory effect was exerted via release of nitric oxide (NO). However, this remains to be shown in conclusive studies.

### Aims/questions

- How do  $\beta$ -adrenoceptor subtypes ( $\beta_1$ -3) contribute to rat bladder relaxation?
- Is nitric oxide involved in the  $\beta$ -adrenoceptor-induced relaxation?
- Is this altered in a state of inflammation?

## RESULTS

The presence of a  $\beta$ -adrenoceptor agonist ( $5 \times 10^{-8}$  M –  $5 \times 10^{-6}$  M) significantly attenuated methacholine-induced bladder contraction, regardless of the selectivity profile of the agonist ( $\beta_1$ ,  $\beta_{1/2}$  or  $\beta_3$ ), causing a right-shift of the concentration-response curve in both healthy and inflamed tissue ( $p < 0.05$  for all comparisons between logEC<sub>50</sub> values for methacholine in the absence of a  $\beta$ -adrenoceptor agonist to logEC<sub>50</sub> values for methacholine in the presence of a  $\beta$ -adrenoceptor agonist;  $n = 6$ ). Inhibition of NO synthase did not significantly shift the methacholine-induced contraction-response curves in the presence of a  $\beta$ -adrenoceptor agonist, neither in healthy or inflamed tissue ( $p$ -values remained  $< 0.05$  for all comparisons between logEC<sub>50</sub> values for methacholine in the absence of a  $\beta$ -adrenoceptor agonist to logEC<sub>50</sub> values for methacholine in the presence of a  $\beta$ -adrenoceptor agonist;  $n = 6$ ).



**Figure 1.** Mean contractile responses (as a percentage of high [K<sup>+</sup>] response) to methacholine of bladder strips from healthy and inflamed bladders in the absence (■, ▲, ▼) and presence (○, □, △) of L-NNA, in the absence of  $\beta$ -adrenoceptor agonist ● and presence of dobutamine (a, b), isoprenaline (c, d) or ZD7114 (e, f). The vertical bars represent standard error of the mean (SEM),  $n=6$  in all groups. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  for statistical comparisons (two-way ANOVA) between methacholine responses in the absence of  $\beta$ -adrenoceptor agonist ● and in the presence of a low concentration of  $\beta$ -adrenoceptor agonist ( $10^{-8}$  M; ■).

## STUDY DESIGN

To avoid problems that may arise when studying relaxation in pre-contracted tissue, we chose to study the indirect relaxatory influence that activation of  $\beta$ -adrenoceptors exerts on muscarinic receptor-induced contraction. A total of 36 adult male Sprague-Dawley rats were used in the current study. Each rat was pre-treated with either cyclophosphamide (CYP; 100 mg/kg i.p.) to induce experimental cystitis or saline (4 mL/kg i.p.), serving as controls. Sixty hours later, the rats were sacrificed and their bladders were excised and full-thickness bladder strips were mounted in an organ bath setup. After an equilibration period, methacholine ( $1 \times 10^{-7}$  –  $3 \times 10^{-4}$  M) was applied and the contractile force was measured in the absence and presence of increasing concentrations of a selective  $\beta$ -adrenoceptor agonist ( $5 \times 10^{-8}$  M –  $5 \times 10^{-6}$  M); either dobutamine ( $\beta_1$ ), isoprenaline ( $\beta_{1/2}$ ) or ZD7114 ( $\beta_3$ ). Further, each series of measurements was performed in the absence or presence of the NO synthase inhibitor L-NNA ( $1 \times 10^{-4}$  M).

## CONCLUSIONS

The current data shows that the presence of a  $\beta$ -agonist, regardless of its subtype selectivity ( $\beta_1$ ,  $\beta_{1/2}$  or  $\beta_3$ ), attenuates muscarinic receptor-induced detrusor contraction. Somewhat surprisingly, this attenuation does not seem to be dependent on release of nitric oxide and remains constant in a state of inflammation. This suggests that treatment against OAB with a  $\beta_3$ -adrenoceptor agonist is suitable also for patients with concomitant cystitis.