

## INTRODUCTION & OBJECTIVE

- $\beta_3$ -adrenoceptors ( $\beta_3$ -ARs) have been reported to predominantly mediate the relaxation of the human isolated urinary bladder (1). Therefore,  $\beta_3$ -ARs represent a potentially important drug target for the treatment of overactive bladder.
- While the contribution of  $\beta$ -ARs on the relaxation of mouse isolated urinary bladder has been shown (2), the role of  $\beta_3$ -ARs in this relaxant effect was not investigated.
- In mouse, in a previous *in vivo* study, we demonstrated an increase of bladder capacity following  $\beta_3$ -ARs agonist administration (CL316,243) suggesting an implication of  $\beta_3$ -ARs on mouse isolated urinary bladder relaxation (3).
- Therefore, the aim of this study was to examine the implication of  $\beta_3$ -ARs on the relaxation and spontaneous motility of mouse isolated urinary bladder by evaluating the effects of CL316,243 and SR 59230A (a selective  $\beta_3$ -ARs antagonist).

## MATERIALS & METHODS

- Female mice (C57Bl6/J, 18-20 g) were sacrificed and the whole urinary bladder excised. Two detrusor muscle strips were obtained and mounted, in 5 mL glass organ bath filled with oxygenated (95 %  $O_2$  - 5 %  $CO_2$ ) Krebs solution (pH = 7.4) kept at 37°C. Tissues were allowed to equilibrate under 0.5 g initial tension. The experiments were conducted in the presence of propranolol (1  $\mu$ M) and prazosin (1  $\mu$ M) in order to block  $\beta_1/\beta_2$ -ARs and  $\alpha_1$ -adrenoceptors, respectively.
- After 60 min of equilibration, SR 59230A (0.3, 1 or 3  $\mu$ M) or its solvent (distilled water) were incubated for 45 min. Then, cumulative concentration-response curves to CL316,243 (0.01-10  $\mu$ M) or its solvent (Krebs solution) were obtained. At the end of the experiments, maximal relaxation was induced with 100  $\mu$ M papaverine. Results were expressed as percentage of relaxation to 100  $\mu$ M papaverine.
- The effects on spontaneous motility of SR 59230A (0.3-10  $\mu$ M) and CL316,243 (0.01-10  $\mu$ M) were evaluated using AUC before and after addition of the drugs. Results were expressed as percentage of variation of spontaneous motility before drug incubation.

## RESULTS

- The solvent for CL316,243 decreased the basal tension with a maximal effect of  $14 \pm 6$  % (data not shown).
- CL316,243, in the range 0.01-10  $\mu$ M, concentration-dependently relaxed the basal tension (Figure 1). The maximal effect, observed at 10  $\mu$ M, was  $66 \pm 4$  %. By using a nonlinear regression, the  $pEC_{50}$  value was  $6.75 \pm 0.18$ .
- SR 59230A caused a statistically significant rightward shift of the curve to CL316,243 in a concentration-dependent manner. The  $pA_2$  value, calculated by Schild plot, was 6.71 (Figure 1).
- CL316,243 decreased (Figure 2) whereas SR 59230A increased (Figure 3) both significantly and in a concentration-dependent manner the detrusor muscle spontaneous motility.

## CONCLUSIONS

- CL316,243 exhibited a concentration-dependent relaxant effect on mouse isolated urinary bladder.
- The antagonist potency of SR 59230A *versus* CL316,243 is similar to the potency reported in mouse isolated colon (4) and in accordance with the affinity for  $\beta_3$ -ARs (5).
- In addition, we found that SR 59230A increased the spontaneous motility of mouse isolated urinary bladder.
- Therefore, this study provides the first evidence for the existence of functional  $\beta_3$ -ARs in the mouse detrusor muscle. These results also suggest that  $\beta_3$ -ARs have an inhibitory physiological role in the detrusor muscle since incubation with the  $\beta_3$  antagonist increased the spontaneous motility of the detrusor muscle.
- In conclusion, these results support the view that selective  $\beta_3$ -ARs agonists could have clinical utility for the treatment of overactive bladder in humans.**

Figure 1: Concentration-response curves for the effects of CL316,243 on basal tension of female mouse isolated urinary bladder in the presence or absence of SR 59230A (n=7-9) and Schild plot for SR 59230A.

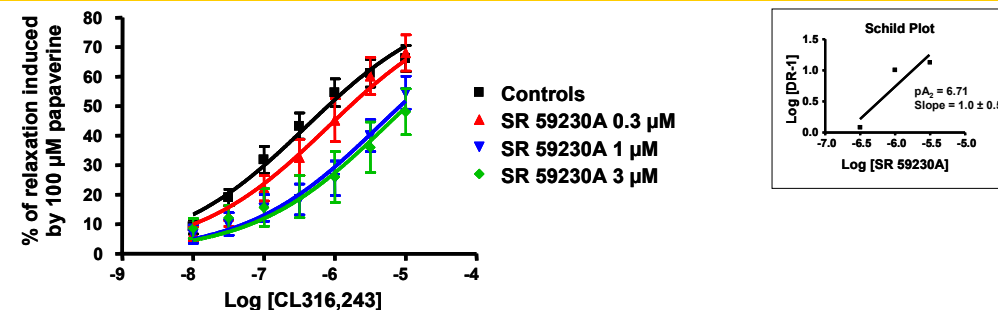


Figure 2 : Effects (expressed as % from values before drug incubation) of solvent (n=9) and CL316,243 (n=8) on the spontaneous motility of female mouse isolated urinary bladder and typical recordings. \*\*p<0.01, \*\*\*p <0.001 vs basal values by one-way ANOVA repeated measures followed by Newman-Keuls test.

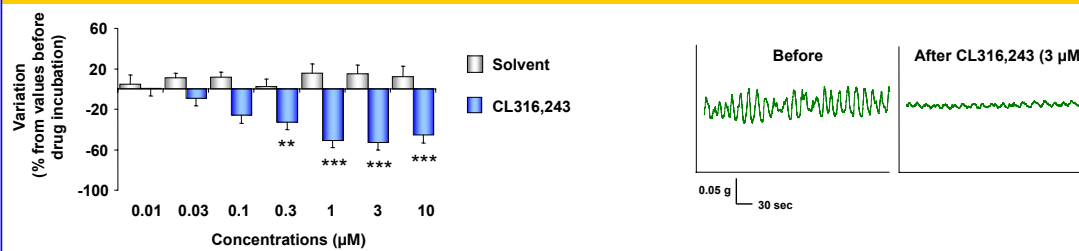
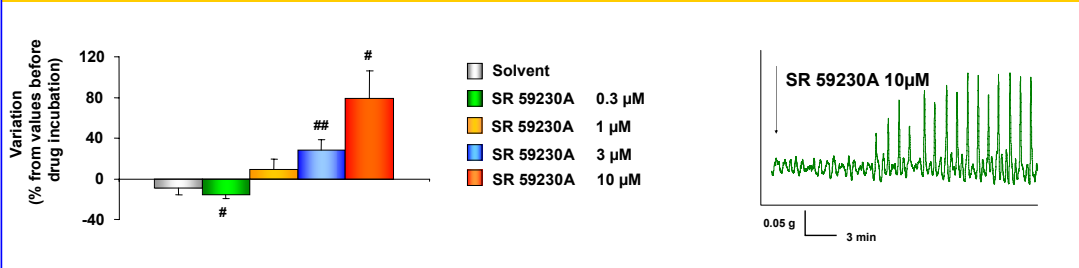


Figure 3 : Effects (expressed as % from values before drug incubation) of solvent (n=8) and SR 59230A (n=7-9) on the spontaneous motility of female mouse isolated urinary bladder and typical recording. #p<0.05, ##p <0.01 vs basal values by paired Student t-test.



## REFERENCES

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