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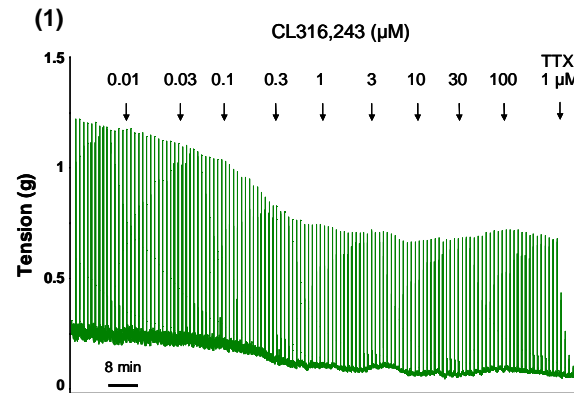
## OBJECTIVES

Since  $\beta_3$ -ARs have been shown to mediate human detrusor muscle relaxation induced by exogenous  $\beta$ -AR agonists, these receptors represent an attractive target for the treatment of overactive bladder (OAB). In previous studies in mice, we showed that the  $\beta_3$ -AR agonist, CL316,243, relaxed basal tone and spontaneous activity of isolated detrusor muscle (1) and increased bladder capacity in anesthetized conditions (2). However, a recent study supported the exclusive role of  $\beta_2$ -ARs in this species (3). The aim of this study was to further elucidate the involvement of  $\beta_3$ -ARs in mouse detrusor muscle relaxation by studying the effects of CL316,243 and two  $\beta_3$ -AR antagonists (SR59230A and L748,337) on contractions induced by electrical field stimulation (EFS).

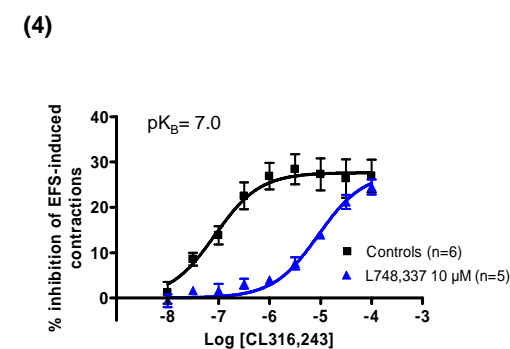
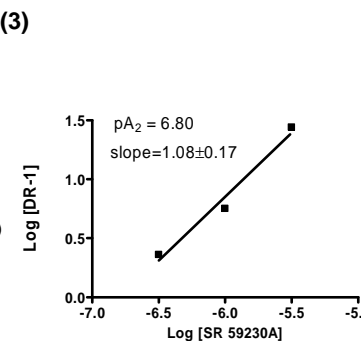
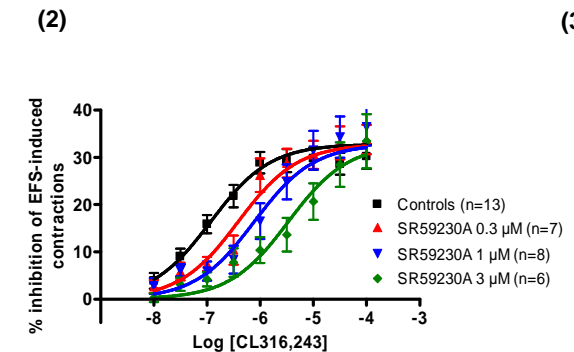
## METHODS

Female C57Bl/6J mice aged 11 weeks (18-23 g) were sacrificed by cervical dislocation. The whole urinary bladder was isolated, freed from connective and fat tissues, then, the dome and the base were removed. Detrusor muscle strips were placed under 0.5 g tension in organ baths filled with a Krebs solution containing propranolol (1  $\mu$ M) and prazosin (1  $\mu$ M) in order to block  $\beta_1/\beta_2$ -ARs and  $\alpha_1$ -ARs, respectively. Bladder contractions were elicited by electrical field stimulation (EFS; 800 mA, pulse duration 0.3 ms, train of pulses 2 s every 60 s) and concentration-response curves (CRCs) to CL316,243 (0.01 to 100  $\mu$ M) were obtained. CRCs were constructed in the presence of SR59230A (0.3, 1, 3  $\mu$ M), or L748,337 (10  $\mu$ M) or their solvents (distilled water and 0.1 % DMSO, respectively). The agonist effects were expressed as % inhibition of basal response to EFS. Using mean values, CRCs to CL316,243 were fitted by nonlinear regression using the GraphPad Prism version 4.0 software to obtain the following parameters: Emax (maximal effect induced by the agonist), pIC<sub>50</sub> (negative logarithm of agonist concentration which induced 50 % of the maximal effect). The antagonist potencies were calculated using the classical Schild plot method (pA<sub>2</sub>) or the following formula: pK<sub>B</sub> = [log antagonist concentration] + log (dose-ratio - 1).

## RESULTS



- A typical recording of the inhibitory effect of CL316,243 on EFS-induced contractions is shown in Figure 1. CL316,243 (0.01 to 100  $\mu$ M) significantly and concentration-dependently inhibited neurogenic contractions.
- pIC<sub>50</sub> values for CL316,243 were  $6.9 \pm 0.1$  and  $7.1 \pm 0.1$ , (in the presence of vehicle for SR59230A and L748,337, respectively) and the maximal inhibitory effects were  $33 \pm 1\%$  and  $28 \pm 1\%$  of the basal response to EFS, respectively (Figure 2).
- SR59230A shifted the CRC of CL316,243 to the right in a concentration-dependent manner generating a pA<sub>2</sub> value of 6.80 and a Schild plot slope very close to unity (Figure 3).
- L748,337 (10  $\mu$ M) also shifted the agonist CRC to CL316,243 to the right resulting in a pK<sub>B</sub> value equal to 7.0 (Figure 4).
- The maximal relaxant effect of CL316,243 was unaffected by both SR59230A and L748,337, suggesting competitive antagonism.
- Tetrodotoxin (TTX) at 1  $\mu$ M completely abolished EFS-induced contractions, confirming their neurogenic origin.



## CONCLUSIONS

We have demonstrated, for the first time, the presence of functional  $\beta_3$ -ARs in the mouse isolated urinary bladder. This conclusion is based on two main observations. First, in our experimental conditions, the selective  $\beta_3$ -AR agonist CL316,243 produced significant effects in the presence of propranolol ( $\beta_{1/2}$ -ARs were blocked). Moreover, the potency values of the selective  $\beta_3$ -AR antagonists SR59230A and L748,337, were similar to affinities of these antagonists at cloned  $\beta_3$ -ARs and to potencies reported in other  $\beta_3$ -AR functional assays in rodents (4-5). It remains unclear whether the reduction of EFS-induced contractions is a postjunctional effect (through a direct action on the smooth muscle), a prejunctional effect (through an inhibition of neurotransmitter release) or a combination of both. Based on the current literature, it seems likely that the effect is primarily postjunctional, however neurotransmitter overflow studies would be required to answer this question.

**We conclude that this experimental model could be useful to screen new  $\beta_3$ -AR agonists for the treatment of OAB in humans.**

## REFERENCES

- 1) Deba *et al.* Eur Urol. **S7**: 182, 2008. 2) Lluel *et al.* J Urol. **177**: 327, 2007. 3) Wuest *et al.* J Pharmacol Exp Ther. **328**: 213-222, 2009. 4) Manara *et al.* Br J Pharmacol. **117**: 435-442, 1996. 5) Candelore *et al.* J Pharmacol Exp Ther. **290**: 649-655, 1999.