

# Stimulation of $\beta_3$ -adrenoceptors inhibits EFS-induced contractions of human isolated urinary bladder

N° Tue 204

Moèz Rekik<sup>1</sup>, Céline Rouget<sup>1</sup>, Philippe Camparo<sup>2</sup>, Henry Botto<sup>3</sup>, Philippe Lluet<sup>1</sup>, Timothy D Westfall<sup>1</sup>, Stefano Palea<sup>1</sup>

1. UROsphere, Faculty of Pharmaceutical Sciences, 35 Chemin des Maraîchers, Toulouse, France 2. Foch Hospital, Anatomy and Cytopathology Department, Suresnes, France 3. Foch Hospital, Urology Department, Suresnes, France

## Objectives

Activation of  $\beta$ -adrenoceptors ( $\beta$ -ARs) has been shown to produce relaxation of pre-contracted detrusor muscle suggesting that  $\beta$ -AR agonism could promote relaxation of the urinary bladder during urine storage.  $\beta$ -ARs are sub-classified into  $\beta_1$ ,  $\beta_2$  and  $\beta_3$ -subtypes. The distribution of  $\beta$ -AR subtypes in the bladder is species dependent. However, in some species including human, the  $\beta_3$ -AR subtype appears to be predominant. In human bladder, this receptor has been shown to mediate relaxation of pre-contracted human detrusor strips<sup>(1-3)</sup> suggesting that  $\beta_3$ -AR agonists have potential for the pharmacological treatment of overactive bladder. A number of studies have demonstrated an effect of  $\beta$ -AR agonists on basal tension and pre-contracted detrusor strips, however very little is known about how  $\beta$ -AR agonism affects neuronal efferent activity.

The aim of the present study was to investigate the effect of  $\beta$ -AR agonism on neurogenic contractions of human isolated detrusor and to characterize the  $\beta$ -AR subtypes involved.

## Methods

- Detrusor smooth muscle strips (devoid of urothelium) were obtained from 11 patients (72  $\pm$  3 years old, 10 male and 1 female) undergoing cystectomy due to bladder carcinoma.
- Strips were mounted in organ baths under 1 g of initial tension and electrical field stimulation (EFS) applied using the following parameters: maximal current, frequency of 10 Hz, square pulses of 0.1 ms, trains of 5 s every min.
- CGP20712 ( $\beta_1$ -AR antagonist at 300 nM), ICI118,551 ( $\beta_2$ -AR antagonist at 30 and 100 nM), L748,337 ( $\beta_3$ -AR antagonist at 0.3, 1 and 3  $\mu$ M), or their respective vehicles were added to the organ bath followed 20 min later by a cumulative concentration-response curve to a  $\beta$ -AR agonists (isoproterenol, L755,507 or CL316,243).
- Responses obtained at each concentration of agonist were expressed as percentage variation from basal EFS-induced contractions obtained before agonist addition.

## Conclusions

While it is well known that stimulation of  $\beta_3$ -ARs produces relaxation of basal tension and pre-contracted strips of human isolated bladder, there are very few reports on the effect of  $\beta$ -AR agonists on EFS-induced responses. Here, we clearly demonstrate that isoproterenol is able to significantly inhibit EFS-induced contractions of human detrusor through activation of  $\beta_3$ -ARs.

In contrast, the human  $\beta_3$ -AR partial agonist L755,507 produced a potent, but only partial inhibition of EFS-responses while the rodent  $\beta_3$ -AR selective agonist CL316,243 only produced a slight effect.

While clearly  $\beta_3$ -AR agonism can produce relaxation of human bladder through post-junctional receptors (and therefore relaxation of basal tension during urine storage), the current results may imply that  $\beta_3$ -AR agonism can also inhibit neuronally mediated acetylcholine release through pre-junctional receptors and thus directly modulate neuronal contractions. Further studies are necessary to confirm this hypothesis.

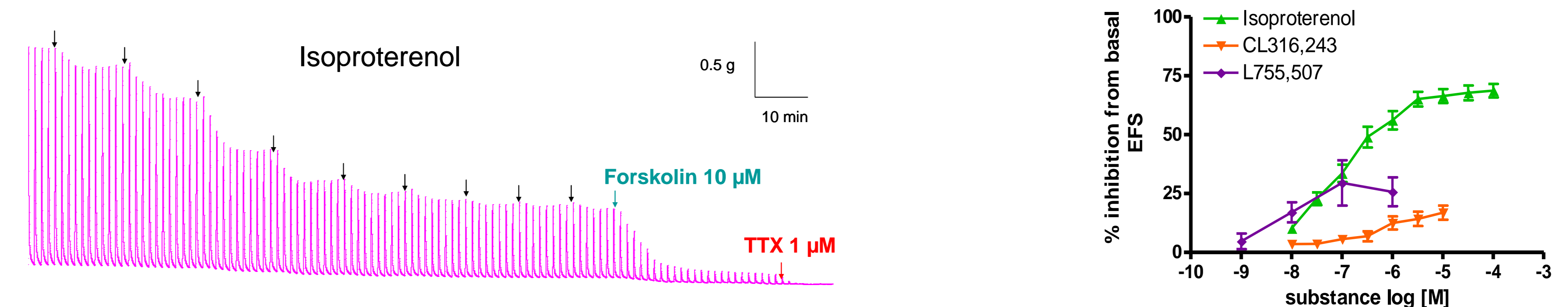
## References

- Wuest *et al*, J Pharmacol Exp Ther, 328:213-222, 2009.
- Yamazaki *et al*, Br J Pharmacol, 124:593-9, 1998.
- Tyagi *et al*, Int Braz J Urol, 35:76-83, 2009.
- Candelore *et al*, J Pharmacol Exp Ther, 290:649-655, 1999.

## Results

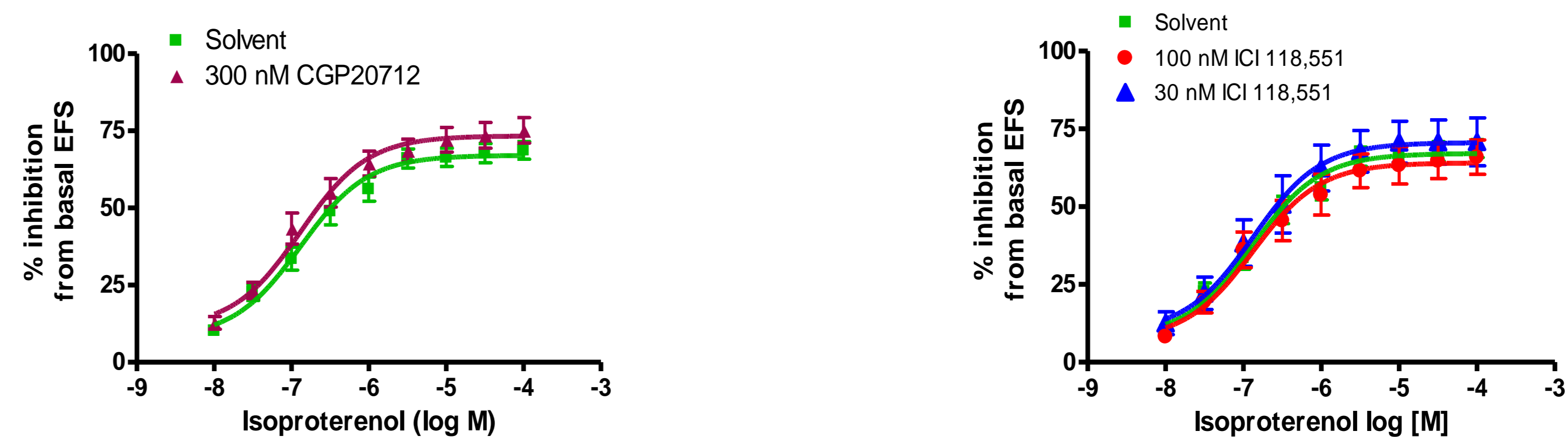
### Effects of $\beta$ -AR agonists on EFS-induced contractions of human detrusor muscle

- Isoproterenol (10 nM to 100  $\mu$ M) inhibited TTX-sensitive EFS-induced contractions of human bladder smooth muscle strips in a concentration-dependent manner with an  $EC_{50}$  value of  $0.19 \pm 0.11 \mu$ M and an  $E_{max}$  value of  $66.6 \pm 3.9\%$  at 30  $\mu$ M.
- In contrast to isoproterenol, the human  $\beta_3$ -AR partial agonist L755,507 produced a potent but a maximal inhibition of  $30.0 \pm 8.9\%$  at 100 nM, while the rodent selective  $\beta_3$ -AR agonist CL316,243 only decreased EFS-induced contractions by  $20.8 \pm 3.5\%$  at 10  $\mu$ M.
- The corresponding solvents had no effects on EFS-induced contractions of human urinary bladder.



### Effects of $\beta_1$ and $\beta_2$ -AR antagonists on isoproterenol-induced inhibition of EFS-induced contractions of human detrusor muscle

- The  $\beta_1$ -AR antagonist CGP20712 (300 nM) had no effect on the response to isoproterenol.
- The  $\beta_2$ -AR antagonist ICI118,551 (30 and 100 nM) also had no effect on the response to isoproterenol.



### Effects of a $\beta_3$ -AR antagonist on isoproterenol-induced inhibition of EFS-induced contractions of human detrusor muscle

- The  $\beta_3$ -AR antagonist L748,337 produced a concentration-dependent rightward shift of isoproterenol-induced response curves without affecting the maximal effect.
- Schild plot analysis revealed a  $pA_2$  value of 7.37 and a slope of  $1.07 \pm 0.19$  indicating competitive antagonism.
- The  $pA_2$  value for L748,337 is lower than the one previously reported in human recombinant  $\beta_3$ -AR expressed in CHO cells (8.40)<sup>(4)</sup> but similar to the value ( $pK_B = 7.65$ ) reported in human isolated urinary bladder precontracted with carbachol<sup>(1)</sup>.

