The phosphodiesterase type 4 inhibitor, rolipram, is more efficient to relax detrusor smooth muscle in rats with overactive bladder than in control rats

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OBJECTIVES

Detrusor relaxation is mainly mediated by the cAMP pathway, which is activated through stimulation of adenylyl cyclase via the fixation of noradrenaline to β-adrenoceptors [1]. At the cellular level, the intensity and the duration of the intracellular cAMP and cGMP signals are partly regulated by the phosphodiesterase (PDE) enzymes whose functions are to degrade cyclic nucleotides into their inactive metabolites. Among the 11 distinct PDE families described, PDE4 is selective for cAMP hydrolysis, and it has been widely evidenced that the elevation of cAMP levels by PDE4 inhibition relaxes various types of smooth muscle fibers [2] .

1 Andersson KE, et al; Physiol Rev 2004;84:935–86
2 Conti M, et al; J Biol Chem 2003;278:5493–6.

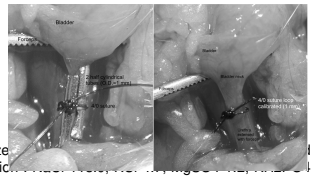
The aim of this study was to evaluate the effect of the PDE4 inhibitor rolipram on detrusor smooth muscle tone in a rat pathological model of overactive bladder (OAB) i.e with partial bladder outlet obstruction (BOO)

MATERIALS & METHODS

Animal preparation

Female Wistar rats (200-225 g) were anesthetized with isoflurane (1-1.2%, Centravet, Plancoët, France). The bladder and the proximal urethra of all rats were exposed through a lower midline abdominal incision and were free from surrounding connective tissue. An extraurethral metal rod with an external diameter of 1 mm, was placed around the urethra to provide a calibrated loop for a single 4-0 silk suture inducing a partial bladder outlet obstruction (obstructed rats, N=7). The metal rod was removed after calibrated urethral ligation and the abdominal wall was closed. Sham rats underwent the same surgical procedure without urethral ligation (non-obstructed rats, N=8). 6 weeks of partial bladder outlet obstruction, rats were anesthetize[illegible] and placed in Krebs-HEPES buffer (with the following millimolar composition [illegible] 1.2; CaCl2 2.5; NaHCO3 4.2; glucose 11.1; HEPES 20.8; pH 7.4). The bladder dome was isolated from the trigone region and four longitudinal strips were then mounted isometrically in 5 ml organ baths filled with Krebs-HEPES buffer maintained at 37°C and continuously bubbled with 95%O2-5%CO2.

Surgical procedure to perform partial BOO



Bladder strip preparation

In vitro contractile studies

The strips were progressively stretched to 1g and for an equilibration period of 90 min. Following equilibration, contractile reactivity of the strips is evaluated with an exposition to KCl (100 mM, 10 min) and a priming period is achieved with carbachol (3.10^{-5} M, 10 min). Strips were pre-contracted with carbachol 10^{-6} M, and allowed to equilibrate during 30 min. The strips were then pre-treated (30 min) with forskolin (FSK), an activator of adenylyl cyclase, at a concentration (3.10^{-7}M) which does not induce a relaxation by itself, or its vehicle before performing concentration-response curves (CRC) with rolipram or its vehicle (DMSO, final concentrations approximately <0.0005% up to 10^{-6} M) were then performed. At the end of the CRC, strips were exposed to the maximally-effective concentration of forskolin (3.10^{-5} M) to induce maximal relaxation.

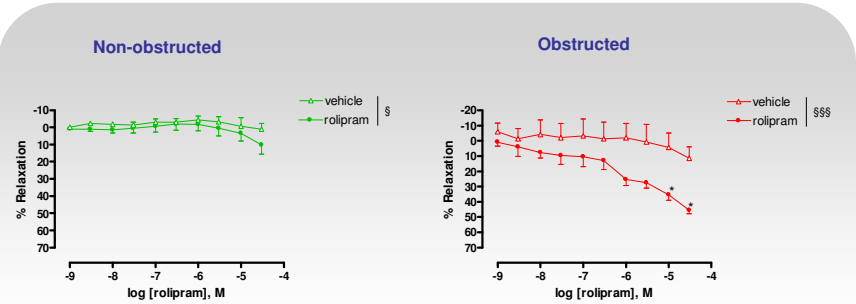
Data analysis

Relaxations were expressed as a percentage of the steady-state tension obtained after the addition of carbachol considered as the maximal contraction. The amount of relaxation produced by forskolin was taken as 100% relaxation. For each CRC in presence of rolipram, a pD2 value (-log concentration of compound that produces 50 % reduction of the maximal response) and a mean maximal effect (Emax) were evaluated in each experimental condition.

Data are expressed as mean ± SEM. Statistical analysis are performed with GraphPad Prism® software.

RESULTS

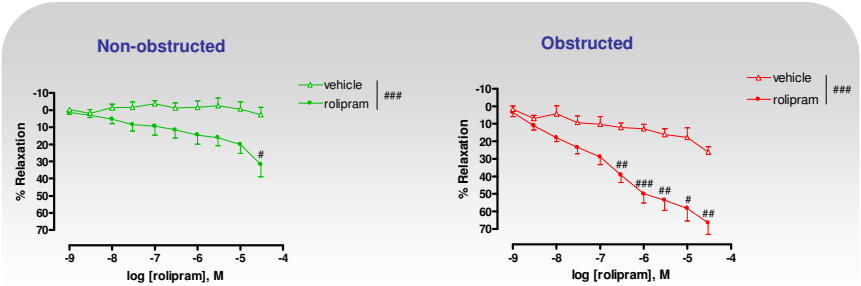
Effect of rolipram on carbachol-induced contractions in detrusor smooth muscle from non-obstructed and obstructed rats



*§p<0.05, §§§p<0.01, two-way ANOVA followed by Bonferroni post-test, *p<0.05*

➢Rolipram is more efficient to relax carbachol-induced contractions of detrusor from obstructed rats than from non obstructed rats

Effect of rolipram on carbachol-induced contractions in detrusor smooth muscle from non-obstructed and obstructed rats in presence of forskolin



###p<0.001, two-way ANOVA with interaction followed by a modified Student's t-test with the Bonferroni's adjustement for multiple comparisons, #p<0.05; ##p<0.01; ###p<0.001

➢The presence of forskolin significantly enhanced the relaxing effect of rolipram both in non-obstructed and non obstructed rat

	Non-obstructed rats (N=8)		Obstructed rats (N=7)	
Forskolin pre-treatment	-	+	-	+
pD_2	could not be determined	5.7 ± 0.4	5.9 ± 0.2	6.6 ± 0.1
Emax (%)	10.1 ± 5.5	31.8 ± 7.1[a]	45.5 ± 2.2[b]	66.6 ± 6.5[a, c]

Significance: ap<0.05 as compared to non obstructed or obstructed rats without forskolin pre-treatment; bp<0.01 as compared to non-obstructed rats without forskolin pre-treatment; cp<0.05 as compared to non-obstructed rats with forskolin pre-treatment, unpaired student t-test analysis.

CONCLUSIONS

- **The PDE4 inhibitor rolipram is more efficient in relaxing carbachol pre-contracted detrusor strips in rats with partial BOO than in control rats.**
- **These results suggest a change in the control of the cAMP pathway in rats with overactive bladder due to BOO.**
- **PDE4 inhibitors deserve further investigation for the treatment of OAB.**

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