Abstract # 194

Influence of the inhibition of cyclic nucleotide phosphodiesterase type 4 on human detrusor smooth muscle contractions

S. Oger [1], D. Behr-Roussel [1], J. Bernabe [1], P. Denys [2], E. Comperat [3], T. Lebret [4], F. Giuliano [1,2]

(1) Pelvipharm, Gif Sur Yvette, (2) Neuro-Urology Unit Dept. of Physical Medicine and Rehabilitation Raymond Poincaré Hospital, Garches, (3) Pitié Salpetrière Hospital, Dept. of Urology, Paris, (4) Foch Hospital, Dept. of Urology, Suresnes, (5) Raymond Poincare Hospital, Dept. of Neurological Rehabilitation, Garches, France

OBJECTIVES

➢ The second messenger, cAMP, controls many physiological processes including smooth muscle relaxation. At the cellular level, the intensity and the duration of the cAMP signal is partly regulated by the phosphodiesterase type 4 (PDE4) enzyme which selectively hydrolyzes cAMP [1]. Elevation of cAMP levels by PDE4 inhibition relaxes various types of smooth muscle cells [2, 3, 4].

➢ In human detrusor smooth muscle, the cAMP pathway plays a critical role in mediating relaxation and PDE4 expression has been characterized [5, 6].

The aim of this study was to evaluate the effect of the selective inhibition of PDE4 activity using the archetypal PDE4 inhibitor, rolipram, on human detrusor strips contractions induced by carbachol

MATERIALS & METHODS

Human bladder strip preparation

Bladders samples were obtained from donors undergoing cystectomy for bladder cancer with no known bladder dysfunction according to their medical chart. Serosal and mucosal layers were removed from the bladder sample, and detrusor strips were mounted in 5 ml organ baths filled with Krebs-HEPES buffer maintained at 37°C and continuously bubbled with 95%O_2-5%CO_2. The strips were connected to force transducers for isometric tension recordings (Pioden Controls Ltd, UK). Following amplification, the tension changes were computerized via MacLab™/8 using Chart™ 5 software (AD Instruments Ltd).

In vitro contractile experiments

The detrusor strips were progressively stretched to 500 mg. Following an equilibration period, contractile reactivity of the strips was evaluated with an exposition to KCl (100 mM, 10 min) and a priming period was achieved with carbachol (3.10^{-6} M, 10 min). Strips were then washed and pre-contracted with a sub-maximal concentration of carbachol (10^{-6} M) and allowed to re-equilibrate during 30 min. For the forskolin (a non selective activator of adenylyl cyclase) pre-treatment, the strips were exposed to forskolin (25 min, 3.10^{-7} M, i.e. concentration giving 15-20% relaxation, as determined in preliminary experiments, data not shown) or vehicle. Then, concentration-response curves to rolipram or vehicle were performed. At the end of the concentration-response curve, strips were exposed to the maximally-effective concentration of forskolin (3.10^{-5} M) to induce maximal relaxation.

Relaxations in response to increasing and cumulative concentrations of rolipram or vehicle were expressed as a percentage of the steady-state tension obtained after the addition of carbachol (10^{-6} M, 30 min) considered as the maximal contraction. The amount of relaxation produced by the maximally-effective concentration of forskolin (3.10^{-5} M) determined at the end of each experiment and after 20 min of stabilization was taken as 100% relaxation.

RESULTS

Effect of rolipram on human bladder strips precontracted with carbachol

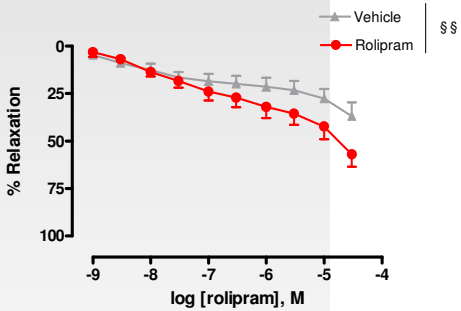

Figure 1 *: Human bladder strips were pre-contracted with carbachol (10^{-6}M). Values are means ± SEM, N=5 experiments with 5 different bladder samples from 5 different donors, Comparisons of the curves are performed with a two-way ANOVA analysis , §§P<0.01.*

	Rolipram (N=5)
pD_2	6.6 ± 0.3
Rmax (3.10^{-5} M)	57 ± 6.5 %

➢ The cumulative addition of rolipram induced a significant concentration-dependent inhibition of human detrusor smooth muscle strip contractions elicited by carbachol

Effect of rolipram on human bladder strips precontracted with carbachol in presence of forskolin

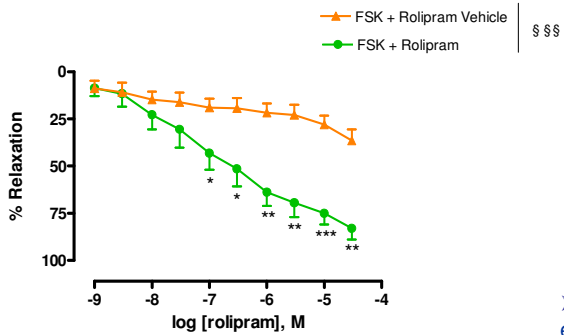

Figure 2 *: The relaxant properties of rolipram or vehicle were evaluated on carbachol-evoked bladder strip contractions in presence of forskolin (FSK), 3.10^{-7}M. Values are means ± SEM, N=5 experiments with 5 different bladder samples from 5 different donors, Comparisons of the curves are performed with a two-way ANOVA analysis, §§§P<0.0001, followed by a modified Student's t-test with the Bonferroni's adjustement for multiple comparisons because of a significant interaction between the two factors (i.e. effect of rolipram and the concentration tested) (*P<0.05 **P<0.01 and ***P<0.001).*

	Forskolin pre-treatment + rolipram (N=5)
pD_2	6.9 ± 0.2
Rmax (3.10^{-5} M)	83 ± 5.7 %

➢ Forskolin pre-treatment dramatically enhanced the concentration-relaxant effect of rolipram with a significant marked effect at the highest concentrations tested

CONCLUSIONS

➢ Selective inhibition of PDE4 activity by rolipram relaxed human detrusor strips precontracted by carbachol.

➢ The elevation of the intracellular cAMP levels by forskolin strongly strengthened the relaxant effect exerted by rolipram.

PDE4 inhibitors could represent an attractive target for the treatment of overactive bladder although it requires further investigation

1. Conti M, et al. Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signaling. *J Biol Chem* 2003;278:5493-5496.
2. Barnette MS, et al. Initial biochemical and functional characterization of cyclic nucleotide phosphodiesterase isozymes in canine colonic smooth muscle. *J Pharmacol Exp Ther* 1993;264:801-812.
3. Torphy TJ, et al. Identification, characterization and functional role of phosphodiesterase isozymes in human airway smooth muscle. *J Pharmacol Exp Ther* 1993;265:1213-1223.
4. Oger S, et al. Anti-inflammatory and utero-relaxant effects in human myometrium of new generation phosphodiesterase 4 inhibitors. *Biol Reprod* 2004;70:458-464.
5. Truss MC, et al. Cyclic nucleotide phosphodiesterase (PDE) isoenzymes in the human detrusor smooth muscle. II. Effect of various PDE inhibitors on smooth muscle tone and cyclic nucleotide levels in vitro. *Urol Res* 1996;24:129-134.
6. Truss MC, et al. Cyclic nucleotide phosphodiesterase (PDE) isoenzymes in the human detrusor smooth muscle. I. Identification and characterization. *Urol Res* 1996;24:123-128.

PELVI PHARM