

Rho-kinase inhibition relaxes detrusor from neurogenic patients

S. Oger¹, D. Behr-Roussel¹, D. Gorny¹, J. Bernabé¹, E. Comperat², E. Chartier-Kastler², P. Denys³, F. Giuliano^{F3,4}

(1) Pelvipharm, Orsay, (2) Pitié Salpêtrière Hospital, Dept. of Urology, Paris, (3) Raymond Poincaré Hospital, Dept. of Neurological Rehabilitation, Garches, France, (4) EA 4501 Université Versailles Saint Quentin en Yvelines

OBJECTIVES

Rho-kinases have a central role in the regulation of bladder smooth muscle from human and various animal species. *In vitro* or *in vivo* data from animal models of overactive bladder (OAB) indicate that rho-kinases could be involved in the pathophysiology of OAB. To date, the role of rho-kinases in patients with neurogenic detrusor overactivity has not yet been explored.

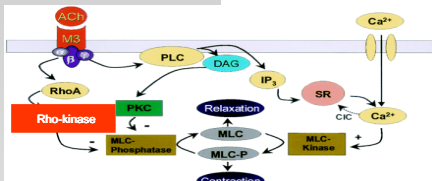


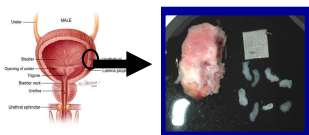
Figure 1: Transmitter signal pathways involved in activation of detrusor contraction via M3 receptors (from Andersson et al, 2004)

We aimed to evaluate the effect of Rho-kinase inhibition on detrusor from neurogenic patients pre-contracted with either carbachol or KCl.

MATERIALS & METHODS

Human detrusor strip preparation

Bladder samples were obtained from 13 different neurogenic patients who underwent partial or total cystectomy. Detrusor strips with or without urothelium were mounted isometrically at a resting tension of 500 mg in a 5 ml organ bath filled with Krebs-HEPES buffer maintained at 37°C and bubbled with 95%O₂-5%CO₂. 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).



The experiments, collection and use of any tissue or other samples are carried out in accordance with the Research Plan, all relevant laws, regulations and codes of practice, including having obtained informed consent of patients in writing.



The strips were equilibrated for 90 minutes. Then, concentration-response curves (CRC) for the rho-kinase inhibitor, Y-27632, (from 10⁻⁸M to 3.10⁻⁵M) or vehicle were generated on either carbachol (1µM)- or KCl (50 mM)-precontracted detrusor (N=7 in each condition). Results were expressed as a percentage of inhibition of the contractile response to carbachol or KCl.

Data Analysis

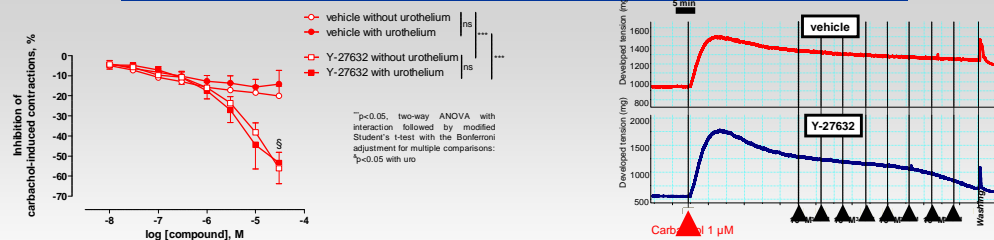
For the evaluation of the effect of drugs to inhibit carbachol or KCl-induced contractions, relaxations in response to increasing and cumulative concentrations of Y-27632 or corresponding concentrations of vehicle are expressed as the percentage of inhibition of the contractile response to carbachol. Data were expressed as mean ± SEM for N experiments corresponding to N = bladder samples obtained, from N different patients.

For each concentration response curves (CRCs) to Y-27632, a pD₂ value (-log [EC50]) where EC50 is the concentration of drug that produces 50% of the maximum effect) was determined using the four parameter logistic model. The maximal effect value (Emax, maximum response that can be produced by the highest concentration of the drug used) was also determined.

Statistical comparisons of CRCs were performed using a two-way ANOVA statistical analysis test followed by Bonferroni post hoc test. In case of significant interaction between two factors, the comparison of CRC was examined at each concentration by a modified Student's t-test with the Bonferroni adjustment for multiple comparisons. Statistical comparisons of the pD₂ and the Emax were performed with the extra sum of squares F-test principle and with a Student's t-test respectively. P values < 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism® 5.02 software.

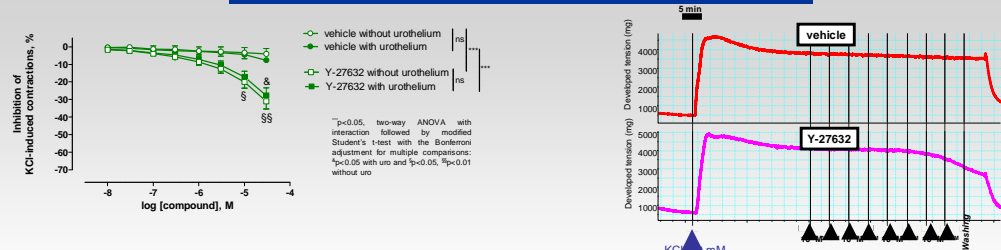
RESULTS

Effect of Y-27632 on carbachol-induced detrusor contraction



Y-27632 induced a significant concentration-dependent inhibition of carbachol-induced contraction of human detrusor strips with or without urothelium. The presence of urothelium did not modify the inhibitory effect of Y-27632 on carbachol-precontracted detrusor strips.

Effect of Y-27632 on KCl-induced detrusor



Y-27632 significantly inhibited KCl-induced contraction of human detrusor strips with or without urothelium. The presence of urothelium did not modify the inhibitory effect of Y-27632 on KCl-precontracted detrusor strips.

Pre-contraction	Y-27632		Statistical analysis (without vs with urothelium)	
	Without urothelium	With urothelium		
Carbachol	n	7	-	
	pD ₂	5.3±0.1	5.5±0.1	ns, extra sum of squares F-test principle
	Emax	-56.1±8.0	-53.4±10.5	ns, t-test
KCl	n	6	7	
	pD ₂	5.38±0.1	5.33±0.1	ns, extra sum of squares F-test principle
	Emax	-31.0±4.34	-27.6±4.4	ns, t-test

Y-27632 was less efficient on KCl-induced contraction than on carbachol-induced contraction whereas its potency was equivalent.

CONCLUSIONS

- Rho-kinases inhibition decreases detrusor contractions from neurogenic patients.
- The mechanisms responsible for this inhibition are different depending on whether the contraction is induced by carbachol or KCl, involving different signaling pathways.
- This supports further investigations regarding the potential development of rho-kinase inhibitors for the treatment of OAB.