Rho-kinase inhibition relaxes detrusor from neurogenic patients

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OBJECTIVES

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

RESULTS

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.

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.

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.

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 (Piden 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 obtaining 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^{-8} M to 3.10^{-6} M) 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 concentration of Y-27632 or corresponding concentrations of vehicle were 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 observed, from N different patients.

For each concentration-response curve (CRC) 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 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.

Abstract # 269

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.