

## EFFECTS OF AN ANTI-MUSCARINIC AGENT AND AN ALPHA-1 RECEPTOR ANTAGONIST ON URINARY ATP LEVELS AND BLADDER ACTIVITY AFTER BLADDER STIMULATION IN RATS

### Hypothesis / aims of study

It is known that bladder epithelial cells express muscarinic receptors and alpha-1 adrenergic receptors and secrete both acetylcholine and adenosine triphosphate (ATP), while bladder sensory nerve endings express muscarinic and purinergic receptors. An alpha-1 receptor antagonist has been reported to inhibit ATP secretion by the bladder epithelium *in vitro*. Therefore, both anti-muscarinic agents and alpha-1 receptor antagonists may possibly inhibit ATP secretion from the bladder epithelium, and this effect could be one of the reasons for improvement of collecting disorders by these agents. In the present study, we examined the effects of propiverine hydrochloride (an anti-muscarinic agent) and naftopidil (an alpha-1 receptor antagonist) on cystometry parameters and the urinary ATP level before and after bladder stimulation in order to investigate the influence of these agents on ATP secretion by the bladder epithelium.

### Materials and methods

Thirty-six female Sprague-Dawley rats were used in this study. The rats were divided into three groups, which were a control group (n = 12), a propiverine group (n = 12), and a naftopidil group (n = 12). Rats from the propiverine and naftopidil groups were given intragastric propiverine hydrochloride or naftopidil dissolved in distilled water (both at 5 mg in 1 ml) by gavage via a catheter once a day without anesthesia. The doses of both agents corresponded to 20-50 times the clinical dose levels (propiverine hydrochloride: 10-40 mg once a day; naftopidil: 25-75 mg once a day). Rats from the control group were administered 1 ml of distilled water by the same procedure. After 2 weeks of treatment, 15 rats (5 from each group) were anesthetized with urethane and a small-bore catheter was inserted into the bladder through the urethra to perform continuous cystometry. Physiological saline was infused into the bladder (0.05 ml/min) via the catheter and bladder activity was monitored. After cystometry was done with physiological saline, the rats also underwent continuous cystometry with a 0.1% acetic acid solution. Cystometry was performed for at least 60 min with each solution, and the changes of bladder activity were recorded. A urine sample was collected from the remaining 21 rats (7 from each group). Each rat was taken out of the cage and placed on a clean board, and the spontaneously voided urine was collected carefully. After that, the rats were anesthetized with halothane and 0.1% acetic acid solution was infused into the bladder for 10 min via a urethral catheter. The animals received a subcutaneous injection of 100 mg of cefazolin sodium hydrate to prevent urinary tract infection. Spontaneously voided urine was also collected at 2-6 hours (day 0) after recovery from halothane anesthesia, and at 1-7 days after bladder stimulation. During this period, administration of distilled water or drugs was continued once a day. The urinary ATP level was measured, and was compared between before and after bladder stimulation in each group. Results are reported as the mean  $\pm$  standard deviation (SD). Student's unpaired t-test was used for statistical analysis, and  $p < 0.05$  was considered to indicate statistical significance.

### Results

During continuous cystometry with physiological saline, there were no significant differences of any of the cystometric parameters among the 3 groups. When cystometry was done with dilute acetic acid, the interval between bladder contractions was significantly shorter and the amplitude of contractions was significantly larger than during cystometry with physiological saline in the control group. In the naftopidil group, the amplitude of bladder contractions was also significantly larger during cystometry with dilute acetic acid than during cystometry with physiological saline. In the propiverine group, however, there were no significant differences of any of the cystometric parameters between before and after infusion of the 0.1 % acetic acid solution. Before the infusion of dilute acetic acid into the bladder, the urinary ATP level (2-8 mol/mg Crex E-10) did not differ among the 3 groups. After bladder stimulation, the urinary ATP level of the control group showed a significant increase to  $851 \pm 568$  mol/mg Crex E-10 on day 0, and  $275 \pm 349$  mol/mg Crex E-10 on day 1. The urinary ATP level of the naftopidil group also showed a significant increase to  $689 \pm 922$  mol/mg Crex E-10 on day 0, and  $58 \pm 75$  mol/mg Crex E-10 on day 1. In the propiverine group, the urinary ATP level increased significantly to  $336 \pm 287$  mol/mg Crex E-10 on day 0, and was  $64 \pm 56$  mol/mg Crex E-10 on day 1. The urinary ATP level of each group returned to baseline at 7 days.

### Interpretation of results

Administration of propiverine or naftopidil without bladder stimulation did not influence the cystometric parameters or the urinary ATP level. Bladder stimulation by acetic acid induced frequent urination and high-amplitude bladder contractions in the control group. However, administration of naftopidil inhibited urinary frequency, and administration of propiverine did not change any of the cystometric parameters. Bladder stimulation by acetic acid increased the urinary ATP level in the control group, but propiverine inhibited this increase of urinary ATP. Naftopidil also slightly inhibited the increase of urinary ATP levels. These results suggest that the dosage of the two drugs were not high enough to influence normal bladder function. However, both drugs inhibited bladder overactivity and the increase of urinary ATP levels (more or less), suggesting that their doses were high enough to influence pathological bladder dysfunction.

Concluding message

The inhibitory effect of propiverine and naftopidil on the bladder may be partly due to blocking ATP release from the bladder epithelium. An increase of the urinary ATP level can be used as a marker for activation of the bladder urothelium.

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**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by Animal care and use committee, Faculty of Medicine, University of the Ryukyus.