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EFFECTS OF AN ANTI-MUSCARINIC AGENT AND AN ALPHA-1 RECEPTOR ANTAGONIST ON PLASMA MONOAMINE LEVELS, URINARY ATP LEVEL 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. Anticholinergic drugs and alpha1 blocking agents are also known to act on the autonomic nervous system. In the present study, therefore, we examined the effects of solifenacin succinate (an anti-muscarinic agent) and tamsulosin hydrochloride (an alpha-1 receptor antagonist) on plasma monoamine levels, urinary ATP level and cystometric parameters before and after bladder stimulation.

Materials and methods
Seventy-two female Sprague-Dawley rats were used in this study. The rats were divided into three groups, which were a control group (n = 24), a solifenacin succinate group (n = 24), and a tamsulosin hydrochloride group (n = 24). Rats from the solifenacin and tamsulosin groups were infused with solifenacin (100 μg/kg/h) or tamsulosin (3 μg/kg/h) via subcutaneously implanted Alzet osmotic minipumps. These doses correspond to about 20 times the human dosage. Rats from the control group were infused with distilled water by the same procedure. After 2 weeks of treatment, we performed 3 examinations as following; Study 1: Twenty-one rats (7 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. Study 2: Other 21 rats (7 from each group) were taken out of the cage and placed on a clean board, and the spontaneously voided urine was collected from each rat, carefully. After that, the rats were anesthetized with halothane and 0.1% acetic acid solution (1 mL) 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 4-6 hours (day 0) after recovery from halothane anaesthesia, and at 1-7 days after bladder stimulation. The urinary ATP level was measured, and was compared between before and after bladder stimulation in each group. Study 3: Remaining 30 rats (10 from each group) were anesthetized with urethane, and blood was withdrawn from vena cava to measure the plasma monoamine (adrenaline, noradrenaline, dopamine, and serotonin) levels. Results are reported as the mean ± standard deviation (SD). Student's unpaired t-test was used for statistical analysis, and $p < 0.05$ was considered to indicate statistical significance.

Results
Study 1: 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 acetic acid, the interval between bladder contractions was significantly shorter and the maximum bladder contraction pressure was significantly higher in the control group than those during cystometry with physiological saline. The maximum bladder contraction pressure during cystometry with acetic acid solution was also significantly higher in the tamsulosin group than that obtained with physiological saline. In the solifenacin group, however, there were no significant differences of any of the cystometric parameters between before and after infusion of acetic acid solution. Study 2: Before the infusion of acetic acid into the bladder, the urinary ATP level (2-6 mol/mg Cre×E-10) did not differ among the 3 groups. After bladder stimulation, the urinary ATP level of the control group and the tamsulosin group showed a significant increase to 483 ± 453 mol/mg Cre×E-10 and 542 ± 295 mol/mg Cre×E-10 on day 0, and 131 ± 146 mol/mg Cre×E-10 and 142 ± 121 mol/mg Cre×E-10 on day 1. The urinary ATP level of the solifenacin group also showed a significant increase to120 ± 125 mol/mg Cre×E-10 on day 0, and 39 ± 20 mol/mg Cre×E-10 on day 1, but their increase degree was lower than those in the control group and the tamsulosin group. The urinary ATP level of each group returned to baseline at 7 days. Study 3: There were no significant differences in plasma monoamine levels among 3 groups.

Interpretation of results
Administration of solifenacin or tamsulosin without bladder stimulation did not influence the cystometric parameters or the urinary ATP level, suggesting that the administered dosages of these drugs were not influence normal bladder function. During cystometry with bladder stimulation, tamsulosin prevented urinary frequency, and solifenacin inhibited both urinary frequency and the increase of bladder contraction pressure. Solifenacin also inhibited the increase of the urinary ATP level after bladder stimulation. However, these drugs did not influence the plasma monoamine levels. Therefore, the inhibitory effects of solifenacin but not tamsulosin on the bladder function might be partly due to blocking the increase of ATP release from the bladder epithelium.

Concluding message
Solifenacin may influence pathological bladder dysfunction but not normal bladder function partly by blocking the increase of ATP release from the bladder epithelium. An increase of the urinary ATP level can be used as a marker for activation of the bladder epithelium.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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