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Imidafenacin exerts the antidiuretic effect by enhancing vasopressin-related responses in orally water-loaded rats

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ABSTRACT

Imidafenacin, an antimuscarinic agent for treating overactive bladder, has an antidiuretic effect, but the detailed mechanisms of action remain unclear. The cholinergic and vasopressin systems are known to interact, for example, in the suppression of vasopressin-induced water reabsorption through muscarinic stimulation in the renal collecting duct. We, therefore, investigated whether vasopressin signaling pathway would participate in the antidiuretic effect of imidafenacin. In female Sprague-Dawley rats, urine production was measured by collecting urine from cystostomy chatheter using a Bollman restraining cage for 2 h after drug i.v. injection and water load (25 ml/kg p.o.). Both imidafenacin and a vasopressin V_2 receptor agonist desmopressin acetate (desmopressin) dose-dependently suppressed urine production. The combination of imidafenacin and desmopressin at the minimum effective doses suppressed the urine production more strongly than each alone. Mozavaptan hydrochloride (mozavaptan, 3 mg/kg), a vasopressin V_2 receptor antagonist, completely inhibited the antidiuretic effects of imidafenacin and desmopressin at their respective minimum effective doses. The antidiuretic effect of desmopressin emerged at the maximum antidiuretic dose level (0.1 μg/kg) even under mozavaptan-treatment, whereas that of imidafenacin (300 μg/kg) was still kept suppressed by mozavaptan. When 300 μg/kg imidafenacin was added to the combination of mozavaptan 3 mg/kg and desmopressin 0.1 μg/kg, the antidiuretic effect was further enhanced. The present study suggests that vasopressin signaling pathway participates in the antidiuretic effect of imidafenacin, and that imidafenacin exerts its antidiuretic effects by enhancing some part of the vasopressin signaling pathway in orally water-loaded rats.

1. Introduction

Nocturia has been defined by the International Continence Society as the complaint that the individual must wake one or more times at night to void (van Kerrebroeck et al., 2002). Because awaking from sleep to void results in sleep loss, daytime fatigue, and mood disturbance, nocturia has a negative impact on health and well-being (van Kerrebroeck and Andersson, 2014), and it is associated with decreased quality of life (Kupelian et al., 2012). The main pathophysiological causes for nocturia are nocturnal polyuria, global polyuria, diminished nocturnal bladder capacity, and sleep impairment, and the nocturnal polyuria is the most frequent cause (Yazici and Kurt, 2015). The pharmacotherapeutic options now clinically applied to nocturia are desmopressin, *anti*muscarinics, alpha-blockers, 5-alpha reductase inhibitors, serotonin reuptake inhibitors, diuretics, and a combination of these treatments (Yazici and Kurt, 2015). Especially, *anti*muscarinics reduce nocturia by increasing bladder capacity (Weiss et al., 2013).

Imidafenacin is an antimuscarinic agent for overactive bladder (OAB)-treatment. There are several studies demonstrating the efficacy of imidafenacin for nocturia in patients with OAB (Leone Roberti Maggiore et al., 2013; Masumori, 2013). Recent clinical studies have shown that the efficacy of imidafenacin for nocturia is probably due to not only increasing bladder capacity but also improving nocturnal polyuria. Indeed, Wada et al. (2012) reported that treatment with imidafenacin significantly reduced the nocturnal polyuria in OAB patients aged 75 years or over. A stratified analysis of data from a phase III randomized, double-blind, placebo-controlled trial of imidafenacin for OAB patients in Japan demonstrated that imidafenacin reduces nocturnal urine production (Yokoyama et al., 2013). In addition, Yokoyama et al. (2015) showed that nocturnal urine volume was significantly reduced by add-on of imidafenacin 0.1 mg before sleep in patients with lower urinary tract symptoms receiving alpha1-blocker treatment. Imidafenacin has been shown to have an antidiuretic effect in water-loaded rats, and this effect is thought to be beneficial for nocturia

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with nocturnal polyuria (Watanabe et al., 2013; Yokoyama et al., 2013, 2015). However, a detailed mechanism of the antidiuretic effect of imidafenacin and its beneficial influence on the clinical efficacy are still controversial (Watanabe et al., 2013).

Watanabe et al. (2013) reported that since imidafenacin did not affect the plasma antidiuretic hormone (ADH) level in the water-loaded rats, the antidiuretic effect of imidafenacin is independent on ADH. On the other hand, the interaction between cholinergic and vasopressin systems has been reported, as carbachol, a muscarinic receptor agonist, inhibits arginine vasopressin (AVP)-induced increases of osmotic water permeability of renal collecting duct cells (Han et al., 1994; Boone and Deen, 2008; Nedvetsky et al., 2009). Thus, there is a possibility that imidafenacin exerts its antidiuretic effect by acting on the vasopressin signaling pathway, but not increasing the release of vasopressin from the pituitary gland. From this point of view, however, little has been studied about the antidiuretic mechanism of imidafenacin. We, therefore, investigated whether vasopressin signaling pathway would participate in the antidiuretic effect of imidafenacin in orally water-loaded rats.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats (Charles River Laboratories Japan, Kanagawa, Japan), weighing 220–290 g, were housed in a room maintained under controlled conditions of 23 ± 3 °C, 55 ± 15% RH and 12–12 h light-dark cycle. The rats were maintained on a solid diet daily with water ad libitum. All animal care/experiments and the study protocol complied with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and were approved, prior to the study, by the Institutional Animal Ethics Committee at Kyorin Pharmaceutical Co., Ltd. Our facility is certified as an animal testing research institute by the Japan Health Sciences Foundation, established under the jurisdiction of the Japanese Ministry of Health, Labor and Welfare.

2.2. Implantations of the cystostomy and the intravenous catheter

The cystostomy catheter was implanted according to a modification of the method reported by Watanabe et al. (2013). Under anesthesia with midazolam (2 mg/kg i.p.), medetomidine hydrochloride (0.15 mg/kg i.p.), and butorphanol tartrate (2.5 mg/kg i.p.), a polyethylene catheter (PE20; Japan Becton Dickinson, Tokyo, Japan) filled with heparinized saline (100 U/ml) was inserted into the femoral vein for injection of test drugs. The catheter was tunneled subcutaneously and exteriorized at the back of the neck between the scapulae. Next, a midline abdominal incision was made and another polyethylene catheter (PE50; Japan Becton Dickinson, Tokyo, Japan) filled with saline was inserted into the tip of the bladder dome for urine collection. The other end of the catheter was closed, then put subcutaneously in the abdomen and kept there until the measurement of urine production. The abdominal muscle and the skin were then sutured. Rectal temperature was maintained at 37 ± 1 °C with a heat lamp during operation. Each rat received an s.c. injection of 40000 U/rat penicillin G potassium and then was housed in a separate cage.

2.3. Measurement of urine production

Seven to nine days after the surgical procedure, urine production was measured using a Bollman restraining cage according to a modification of the method reported by Watanabe et al. (2013). Under isoflurane anesthesia, the implanted cystostomy catheter was exposed from the abdominal subcutaneous tissue and the urethra was sealed with a polyethylene catheter (size 4 with an inner and outer diameter of 0.8 and 1.3 mm, respectively, Hibiki). After each dosing of the drugs (imidafenacin and desmopressin acetate: 1 ml/kg each, mozavaptan hydrochloride: 0.1 ml/kg, i.v.) and water (25 ml/kg p.o.), rats were placed in Ballman restraining cages to measure urine production by collecting urine from the cystostomy catheter for 2 h. Distigmine bromide and its vehicle (2 ml/kg, p.o.) were orally administered 2 h before the water load.

2.4. Drugs and reagents

Imidafenacin and distigmine bromide (distigmine) were synthesized by Kyorin Pharmaceutical Co., Ltd (Tokyo, Japan). Desmopressin acetate (desmopressin) was obtained from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Mozavaptan hydrochloride (mozavaptan) was obtained from Santa Cruz Biotechnology Inc. (Dallas, Texas, USA). Imidafenacin was dissolved in saline with 1 mol/l hydrochloric acid, and the resultant solution was neutralized with 1 mol/l sodium hydroxide, and then serially diluted with saline to desired concentrations. Desmopressin was dissolved in distilled water (DW) and serially diluted with saline to desired concentrations. Mozavaptan and distigmine were dissolved in *N*,*N*-dimethylformamide (DMF) and DW, respectively. The volume of saline and/or DMF corresponding to each of the volumes in drug-treated groups was injected as a vehicle control. In the preliminary study, we had already confirmed that vehicles used in this study had no influence on the urine production.

2.5. Data analysis

Data are expressed as mean ± S.E.M. The statistical differences between two groups were compared using the Student's *t*-test. The statistical differences among more than two groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test or Tukey test. In all comparisons, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of distigmine, imidafenacin and desmopressin on urine production in orally water-loaded rats

First, to investigate whether the activation of endogenous acetylcholine is related to urine production in orally water-loaded rats, we examined the effect of distigmine, an acetylcholinesterase inhibitor, on urine production. As shown in Fig. 1, the oral administration of 1 mg/kg distigmine alone increased urine production.

Imidafenacin dose-dependently suppressed urine production with the minimum effective dose of 10 μg/kg (Fig. 2). This minimum effective dose level is consistent with the reported dose level by Watanabe et al. (2013), and is not largely different from the minimum effective dose level to increase bladder capacity in urethane-anesthetized rats (Yamazaki et al., 2011; Fukata and Yamazaki, 2016). Imidafenacin produced the maximum effect at 100 μg/kg, and the maximum response was approximately half inhibition of vehicle group (Fig. 2).

Desmopressin, a vasopressin V_2 receptor agonist, dose-dependently inhibited urine production with the minimum effective dose of 0.003 μg/kg (Fig. 3). Desmopressin produced the maximum response at a dose level of 0.03 μg/kg, and the maximum response was, unlike imidafenacin, over 90% inhibition of vehicle group (Fig. 3).