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Characterization of muscarinic receptor binding and inhibition of salivation after oral administration of tolterodine in mice

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Abstract

The current study was undertaken to characterize the effects of oral administration of tolterodine on muscarinic receptor binding in the bladder and submaxillary gland and on salivation in mice. In the in vitro experiment, tolterodine and its metabolite (5-hydroxymethyl metabolite: 5-HM) competed concentration-dependently with [*N*-methyl-^{3}H]-scopolamine ([^{3}H]NMS) in the mouse bladder, submaxillary gland and heart, and the potencies of both agents were greater than that of oxybutynin. After oral administration of tolterodine (6.31, 21.0 μmol/kg) and oxybutynin (76.1 μmol/kg), there was a dose and time-dependent increase in K_d values for specific [^{3}H]NMS binding in the bladder, prostate, submaxillary gland, heart, colon and lung, compared with control values, suggesting significant muscarinic receptor binding in each tissue. The K_d increase in each tissue by oral oxybutynin reached a maximum value of 0.5 h after oral administration and then rapidly declined, while that by tolterodine was greatest 2 h after the administration and it was maintained for at least 6 or 12 h, depending on the dose and on the tissue. Thus, muscarinic receptor binding of oral tolterodine was slower in onset and of a longer duration than that of oxybutynin. Also, oral oxybutynin showed relatively greater receptor binding in the submaxillary gland as compared with other tissues, but such high selectivity to the exocrine gland muscarinic receptors was not observed by oral tolterodine. Oral administration of tolterodine and oxybutynin reduced significantly the pilocarpine-induced salivary secretion in mice, and the attenuation of oral tolterodine appeared more slowly and it was more persistent than that of oral oxybutynin. The antagonistic effect of oral tolterodine on the dose–response curves to pilocarpine was significantly weaker than that of oxybutynin. These data suggest that oral tolterodine, compared with the case of oral oxybutynin, binds more selectively to muscarinic receptors in the mouse bladder than in the submaxillary gland, which may be advantageous in treating patients with overactive bladder.

Keywords: Bladder; Submaxillary gland; Oral administration; Tolterodine; Oxybutynin; Muscarinic receptor binding; Salivation

1. Introduction

Overactive bladder is a highly prevalent disease that negatively impacts the quality of life of diagnosed patients (Wyndaele, 2001). Overactive bladder results from involuntary contractions of the detrusor muscle during bladder filling. Anti-muscarinic agents, such as oxybutynin, are the most common drugs used to treat overactive bladder because normal voiding processes and involuntary detrusor contractions during bladder filling are mediated mainly by muscarinic receptors (Andersson, 1988; Wein, 1990). However, peripheral antimuscarinic adverse events, such as dry mouth, tachycardia and accommodation paralysis, have limited the use of these drugs (Yarker et al., 1995).

Based on previous pharmacological and molecular biological studies, muscarinic receptors are classified into five subtypes (M_1–M_5) (Hulme et al., 1990; Caulfield, 1993). It is known that the heart and salivary gland contain predominantly M_2 and M_3 subtypes, respectively (Giraldo et al., 1988; Caulfield, 1993), whereas the bladder contains both, with the M_2 dominating over the M_3 subtype (Wang et al., 1995). Also, our preliminary data with each subtype knockout mice show that the M_3 subtype is expressed predominantly in the submaxillary gland and moderately in the prostate and bladder, whereas M_2 subtype is the main subtype present in

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the bladder, heart, lung and colon (Oki et al., unpublished observation).

Tolterodine has been developed as a new muscarinic receptor antagonist to treat overactive bladder (Guay, 1999). Pharmacological and radioligand binding studies have shown that tolterodine exerts a potent antimuscarinic effect in the isolated detrusor muscle of guinea pigs and humans (Nilvebrant et al., 1997a). In addition, tolterodine has been demonstrated to display a favorable tissue selectivity for the urinary bladder compared with the salivary glands in cats (Nilvebrant et al., 1997a), but the mechanism whereby this drug causes bladder selectivity has not been adequately elucidated. Tolterodine is extensively metabolized in the liver to form an active metabolite, 5-hydroxymethyl metabolite (5-HM) (Postlind et al., 1998; Nilvebrant et al., 1997b) and the metabolism of tolterodine in mice has similar metabolic routes as humans (Påhlman et al., 2001b). Thus, the effect of tolterodine in mice may predict pharmacodynamic effects of tolterodine in humans. As shown in our previous studies with other drugs (Yamada et al., 2003; Oki et al., 2004), the characterization of muscarinic receptor binding of tolterodine under the influence of various pharmacokinetic and pharmacodynamic factors appears to explain further the pharmacological specificity, in particular because the pharmacological effects caused by oral tolterodine may occur as a result of the summation of unchanged form and 5-HM in tissues. In this study, we have determined muscarinic receptor binding in several mouse tissues including the urinary bladder and submaxillary gland, and also salivary secretion following oral administration of tolterodine at the pharmacologically relevant doses, compared with those of oral oxybutynin.

2. Materials and methods

2.1. Materials

[^{3}H]NMS ([*N*-methyl-^{3}H]scopolamine methyl chloride: 3.03 TBq/mmol) was provided by Perkin Elmer Life Sciences, Inc., Boston, MA., tolterodine tartrate and 5-HM were provided by Pharmacia Co. Ltd., Kalamazoo, MI, USA and oxybutynin hydrochloride was provided by Meiji Milk Products Co. Ltd., Odawara, Japan. All other chemicals were purchased from commercial sources.

2.2. Animals

Male ddY strain mice 9 to 16 weeks of age (Japan SLC Inc., Shizuoka, Japan) were used in this study. Mice were housed with a 12 h light–dark cycle and fed laboratory food and water ad libitum.

2.3. Administration of oxybutynin and tolterodine

Mice were fasted for 16 h and then received oral oxybutynin (76.1 μmol/kg) or tolterodine (6.31, 21.0 μmol/kg) dissolved in distilled water. The control animals received vehicle alone. This study was conducted according to the guidelines approved by the Experimental Animal Ethical Committee of University of Shizuoka.

2.4. Tissue preparation and muscarinic receptor assay

The tissue preparation and muscarinic receptor binding assay using [^{3}H]NMS were performed as previously described (Oki et al., 2004). At 0.5 to 24 h after the drug administration, mice were exsanguinated by taking the blood from the descending aorta under temporary anesthesia with diethyl ether, and the tissues were then perfused with cold saline from the aorta. Then, the bladder, prostate, submaxillary gland, heart, lung and colon were dissected, and fat and blood vessels were removed by trimming. The tissues from 3 mice were pooled for a determination, because of the small amounts of tissue weight. The tissues were minced with scissors and homogenized by Kinematica Polytron homogenizer in 19 volumes of cold 30 mM Na^+/HEPES buffer (pH 7.5). The homogenates were then centrifuged at 40,000 ×*g* for 20 min. The resulting pellet was finally resuspended in cold buffer for the binding assay. In the ex vivo experiment, there was a possibility that oxybutynin and tolterodine might dissociate in part from the receptor sites during tissue preparation (homogenization and suspension) after drug administration. Yamada et al. (1980) have previously shown that the dissociation of antagonists from receptor sites at 4 °C is extremely slow. Therefore, to minimize the dissociation of oxybutynin and tolterodine from the receptor sites, all steps for the preparation were performed at 4 °C. Protein concentrations were measured according to the method of Lowry et al. (1951).

The homogenates (the average protein concentrations for bladder, prostate, submaxillary gland, heart, lung and colon: 300, 100, 200, 770, 360 and 300 μg/assay) of mouse tissues were incubated with different concentrations (0.06 to 1.0 nM) of [^{3}H]NMS in 30 mM Na^+/HEPES buffer (pH 7.5) (incubation volume: 1 ml). Incubation lasted 60 min at 25 °C. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD) through Whatman GF/B glass fiber filters, and the filters were then rinsed 3 times with 3 ml of cold buffer. Tissue-bound radioactivity was extracted from the filters by placing them overnight by immersion in scintillation fluid, and radioactivity was determined by a liquid scintillation counter. Specific [^{3}H]NMS binding was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine. All assays were conducted in duplicate.

2.5. Measurement of salivary secretion

Mice were anesthetized with the intraperitoneal administration of pentobarbital (161 μmol/kg). Immediately after wiping the saliva remaining in the oral cavity with a cotton ball, the whole saliva in the cavity was collected by absorption with 3 to 5 cotton balls for 10 min, and then the cotton balls were immediately weighed on an electric balance to prevent moisture loss. The weight of the secreted saliva was estimated as the difference between the weight of the cotton ball before and after the application in the oral cavity. The effects of oral