Advantages for Transdermal over Oral Oxybutynin to Treat Overactive Bladder: Muscarinic Receptor Binding, Plasma Drug Concentration, and Salivary Secretion

Tomomi Oki, Ayako Toma-Okura, and Shizuo Yamada

Department of Pharmacokinetics and Pharmacodynamics and Center of Excellence Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan

Received August 22, 2005; accepted November 9, 2005

ABSTRACT

To clarify pharmacological usefulness of transdermal oxybutynin in the therapy of overactive bladder, we have characterized muscarinic receptor binding in rat tissues with measurement of plasma concentrations of oxybutynin and its metabolite N-desethyl-oxybutynin (DEOB) and salivation after transdermal oxybutynin compared with oral route. At 1 and 3 h after oral administration of oxybutynin, there was a significant increase in apparent dissociation constant (K_d) for specific [N-methyl-3H]scopolamine ([3H]NMS) binding in the rat bladder, submaxillary gland, heart, and colon compared with control values. Concomitantly, submaxillary gland and heart showed a significant decrease in maximal number of binding sites (B_max) for [3H]NMS binding, which lasted until 24 h. Transdermal application of oxybutynin caused dose-dependent increases in K_d values for specific [3H]NMS binding in rat tissues. The increment of K_d values by transdermal oxybutynin was dependent on the application time. Plasma concentrations of oxybutynin and DEOB peaked at 1 h after oral oxybutynin. In contrast, plasma concentrations of oxybutynin increased slowly, depending on the transdermal application time of this drug until 12 h. Suppression of pilocarpine-induced salivation in rats due to transdermal oxybutynin was significantly weaker and more reversible than that by oral oxybutynin, which abolished salivary secretion. The present study has shown that transdermal oxybutynin binds significantly to rat bladder muscarinic receptors without producing both long-lasting occupation of exocrine receptors and cessation of cholinergic salivation evoked by oral oxybutynin. Thus, the present study provides further pharmacological basis for advantage of transdermal over oral oxybutynin in the therapy of overactive bladder.

An overactive bladder, characterized by symptoms of increased frequency of micturition, urgency, and urge incontinence, is very common in the geriatric population, a group that rapidly increases in number (Wein and Rovner, 2002). Urge urinary incontinence is one of the most common types of incontinence in geriatric patients (Resnick and Yalla, 1985). Anticholinergic agents, such as oxybutynin, are widely used to treat overactive bladder because parasympathetic innervation is the predominant stimulus for bladder contraction (Andersson, 1993). However, the use of oxybutynin is often limited by the systemic side effects, such as dry mouth, blurred vision, constipation, and tachycardia, which present frequently as serious problems in patients receiving oral oxybutynin (Yarker et al., 1995). The therapeutic and unwanted effects of anticholinergic agents in patients with overactive bladder stem from blockade of muscarinic receptors in the bladder and other tissues, respectively. To reduce or even eliminate systemic anticholinergic adverse effects of oxybutynin, novel anticholinergic agents and dosage forms have been currently developed that may exhibit pharmacological selectivity in the bladder relative to the salivary gland (Nilvebrant et al., 1997; Abrams et al., 1998; Anderson et al., 1999; Gupta and Sathyan, 1999; Chapple, 2000; Davila et al., 2001; Ikeda et al., 2002). In fact, it has been shown that the oxybutynin chloride extended-release dosage form (Ditropan XL; ALZA Corp., Palo Alto, CA) exhibits lower rate of dry mouth than the immediate release form of oxybutynin in patients with overactive bladder (Anderson et al., 1999; Sathyan et al., 2001). It is also worth noting that transdermal therapeutic system of oxybutynin significantly improves anticholinergic adverse effects of oral oxybutynin in patients with overactive bladder (Davila et al., 2001; Dmochowski et al., 2003). Although, in the novel dosage form of oxybutynin, low plasma concentration of its active metabolite N-desethyl-oxybutynin (DEOB) has been suggested to contribute to the
low incidence of anticholinergic side effects (Davila et al., 2001; Dmochowski et al., 2003), the underlying pharmacological mechanism for the advantage of transdermal over oral oxybutynin is unknown.

Our previous studies have revealed that comparative characterization of extent and duration of receptor binding at target and nontarget tissues after clinical route of drug administration is an extremely powerful way to elucidate in vivo pharmacological specificity, such as organ selectivity, efficacy, and safety, in relation to the pharmacokinetics and pharmacodynamics (Uchida et al., 1995; Ohkura et al., 1998; Yamada et al., 1998, 1999, 2001; Oki et al., 2004). Therefore, in the present study, we measured simultaneously muscarinic receptor binding in tissues, including the bladder and submaxillary gland, plasma levels of oxybutynin and DEOB, and saliva output in rats after transdermal application of oxybutynin compared with the oral administration.

Materials and Methods

Materials. [N-methyl-3H]Scopolamine methyl chloride ([3H]NSM; 3.03 TBq/mmol) was purchased from DuPont-NEN Co. Ltd. (Boston, MA). Oxybutynin hydrochloride, its active metabolite DEOB, the transdermal therapeutic system for oxybutynin free base (CS-801; Sankyo Co. Ltd., Tokyo, Japan), and its placebo were donated by Meiji Milk Products Co. Ltd. (Odawara, Japan) and Sankyo Co. Ltd. (Tokyo, Japan). Transdermal therapeutic system of oxybutynin is composed of three layers that consist of backing film, matrix adhesive layer (containing oxybutynin free base), and an overlapping release liner strip. This system is applied to the skin after peeling off the tab (Fig. 1). One patch covers an area of 13 cm2 and contains 33.6 μmol (12 mg) of oxybutynin free base. All other chemicals were purchased from commercial sources.

Animals. Male Sprague-Dawley rats (Charles River Japan, Inc., Kanagawa, Japan) weighing approximately 250 to 350 g were used in this study. Rats were housed with a 12-h light/dark cycle and fed laboratory food and water ad libitum.

Administration of Oxybutynin and DEOB. For oral administration of oxybutynin, rats were fasted for 16 h and then given oral oxybutynin hydrochloride (127 μmol/kg) suspended in distilled water. Control animals received vehicle alone. For the transdermal application of oxybutynin, the back hairs of rats were shaved with an electroshaver (BRAUN Dual 500, Braun, Kronberg, Germany) after cutting hair with electric clipper under temporary anesthesia with diethyl ether. On the next day, transdermal oxybutynin (0.5, 1, and 2 patches/body) was applied to the rats with the shaved back and then the rats were covered with Transpore surgical tape and self-adherent wrap (Coban; 3M Health Care, Tokyo, Japan). The vehicles were applied transdermally to the control rats. Furthermore, oxybutynin (7.61 μmol/kg) and DEOB (8.20 μmol/kg) were injected to the femoral vein of rats under temporary anesthesia with diethyl ether.

Fig. 1. Transdermal therapeutic system of oxybutynin. Transdermal therapeutic system of oxybutynin is composed of three layers that consist of backing film, matrix-after-adhesive layer (containing oxybutynin free base), and an overlapping release liner strip. One patch covers an area of 13 cm2 containing 33.6 μmol (12 mg) of oxybutynin free base. Oxybutynin was transdermally administered to the shaved backs of rats after peeling off the tab.

Tissue Preparation. At various times (oral, 1–24 h; transdermal, 2–24 h-application; intravenous, 1 h) after drug administration, rats were killed 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. The bladder, submaxillary gland, heart, and colon then were dissected, and fat and blood vessels were removed by trimming. The tissues were minced with scissors and homogenized in a Kinematica Polytron homogenizer in 19 volumes of ice-cold 30 mM Na+/HEPES buffer (pH 7.5). The homogenates were then centrifuged at 40,000g for 20 min. The resulting pellet was finally suspended in buffer for the binding assay. In the ex vivo experiment, there was a possibility that oxybutynin 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 was extremely slow. Therefore, to minimize the dissociation of oxybutynin from the receptor sites, all steps in the preparation were performed at 4°C. In fact, there was no large difference in [3H]NMS binding parameters [apparent dissociation constant (Kd) and maximal number of binding sites (Bmax)] between single washing and double washing of rat bladder homogenates at 4°C, 3 h after oral administration of oxybutynin hydrochloride (127 μmol/kg). Protein concentrations were measured by the method of Lowry et al. (1951).

Muscarinic Receptor Binding Assay. The binding assay for muscarinic receptors was performed using [3H]NSM as described previously (Oki et al., 2004). The homogenates (70–350 μg of protein) of rat tissues were incubated with different concentrations (0.06–1.5 nM) of [3H]NSM in 30 mM Na+/HEPES buffer (pH 7.5). Incubation was carried out for 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 three times with 3 ml of ice-cold buffer. Tissue-bound radioactivity was extracted from the filters overnight by immersion in scintillation fluid [21 toluene, 11 Triton X-100, 15 g of 2,5-diphenyloxazole, and 0.3 g of 1,4-bis(2-(5-phenyloxazolyl))benzene], and the radioactivity was determined by a liquid scintillation counter. Specific [3H]NSM binding was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine. All assays were conducted in duplicate.

Measurement of Plasma Concentrations of Oxybutynin and DEOB. The measurement of the plasma concentrations of oxybutynin and DEOB was performed in rats after oral administration of oxybutynin hydrochloride (127 μmol/kg) and transdermal oxybutynin (1 patch/body), as described above. After drug administration, rats were killed by the withdrawal of blood from the descending aorta under anesthesia with diethyl ether. Plasma was isolated from blood by centrifugation. The plasma was stored at −80°C until analysis. Concentrations of oxybutynin and DEOB in plasma were determined by gas chromatography (GC) and mass spectrometry (MS). In brief, a plasma sample (0.1–0.5 ml) was mixed with internal standard ([3H]oxybutynin·HCl and [3H]DEOB·HCl) and, after being alkalized with 0.5 ml of 0.5 M carbonate buffer (pH 9.5), it was extracted with 6 ml of n-hexane. After centrifugation at 1500g for 5 min, the supernatant was evaporated to dryness under reduced pressure. The residue was dissolved in 100 μl of CH3CN, and 0.5 to 1 μl was injected into the GC/MS system. The GC/MS system consisted of a 5890 SERIESII gas chromatograph, a 5792 SERIES mass selective detector, a 7673 GC/SFC injector, a VECTRA 486/66U computer, and a LASER JET 4 printer (Hewlett Packard Co., Palo Alto, CA). Chromatographic separation was carried out using a 15 m × 0.25 mm i. d. × 0.25-μm film UA-1 HT (Frontier Lab Ltd., Fukushima, Japan). The carrier gas was helium at a flow rate of 1.0 ml/min. Oven temperature was held at 150°C for 1 min and then programmed from 150 to 220°C at 20°C/min for first ramp, from 220 to 260°C at 10°C/min for second ramp, and from 260 to 300°C at 30°C/min for third ramp. It was held at 300°C for 2 min before returning to the initial starting temperature of 150°C. The injection
temperature was 200°C. Fragmentation was accomplished by electron impact at 70-eV ionizing voltage and 300-μA ionizing current. Selected ion monitoring was performed at m/z 342 (oxybutynin), m/z 355 (internal standard, [3H]oxybutynin), m/z 189 (DEOB), and m/z 200 (internal standard, [3H]DEOB). The limits of detection of oxybutynin and DEOB in plasma were 1.40 and 3.04 nM, respectively.

**Measurement of Salivary Secretion.** Rats were anesthetized with the intraperitoneal administration of pentobarbital (121 μmol/kg). Any saliva remaining in the oral cavity was removed with a cotton ball before measuring the whole saliva collected in the cavity for a 10-min period. The saliva was absorbed into three to five cotton balls for 10 min, and the balls were weighed on an electric balance immediately after the collection period to prevent moisture loss. To examine the effects of oral and transdermal administration of oxybutynin on pilocarpine-induced salivary secretion, pilocarpine (4.09 mol/kg, dissolved in physiological saline) was administered intravenously 1 to 24 h and the whole saliva were collected at 10-min intervals. The weight of whole saliva secreted in the oral cavity was estimated as the difference between weight of cotton ball before and after the intravenous injection of pilocarpine. In addition, the effects of oral and transdermal administration of oxybutynin on salivary secretion induced by cumulative doses (0.04 to 123 μmol/kg i.v.) of pilocarpine in rats given at 5-min intervals were examined.

**Data Analysis.** Analysis of [3H]NMS binding data were performed as described previously (Yamada et al., 1980). The Kd and Bmax for [3H]NMS were estimated by Rosenthal analysis of the saturation data. Statistical analysis of the data were performed by a one-way analysis of variance followed by Dunnett’s test for multiple comparison. The data were expressed as mean ± S.E. Statistical significance was accepted at P < 0.05.

**Results**

**Effects of Oral and Transdermal Administration of Oxybutynin on Muscarinic Receptors in Rat Tissues.** At 1 and 3 h after oral administration of oxybutynin (127 μmol/kg), there was a significant increase in the Kd values for specific [3H]NMS binding in each tissue of rats compared with the control values (Table 1). The increases in the Kd values in the bladder, submaxillary gland, heart, and colon at 1 h were 54.5, 249, 34.1, and 93.4%, respectively, and those at 3 h were 115, 678, 29.3, and 143%, respectively. Thus, the magnitude of enhancement in the Kd values at 3 h produced by oral oxybutynin was significantly (P < 0.01) greater in the submaxillary gland than in other tissues. There was little significant increase in the Kd values in all of these tissues at 12 and 24 h later. In the submaxillary gland and heart of oxybutynin-administered rats, there was a significant (submaxillary gland, 26.7–56.8%; heart, 13.5–19.5%) reduction in the Bmax values for [3H]NMS binding from 1 or 3 to 24 h after oral administration compared with the corresponding control values.

The transdermal application of oxybutynin at 1 patch (33.6 μmol/body for 2, 4, and 12 h brought about a time-dependent increase in the Kd values for specific [3H]NMS binding in each tissue compared with the corresponding control value, and no further increase was seen at 24 h after application (Table 2). The increase in the Kd values was significant after 2 to 24 h of application in the submaxillary gland and colon and after 4 or 12 to 24 h of application in the heart and bladder. The rate of enhancement in the Kd values after transdermal application of oxybutynin for 2 to 24 h was significantly (P < 0.01) greater in the submaxillary gland (226–547%) compared with the bladder (90.4–102%), heart (22.2–36.7%), and colon (38.8–101%). There was little change (226–547%) compared with the bladder (90.4–102%), heart, and colon at 1 and 3 h after oral administration compared with the control values.

**Table 1**

<table>
<thead>
<tr>
<th>Organs</th>
<th>Time after Oral Oxybutynin</th>
<th>Kd</th>
<th>Bmax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>pM</td>
<td>fmol/mg protein</td>
</tr>
<tr>
<td>Bladder</td>
<td>Control</td>
<td>167 ± 5</td>
<td>158 ± 7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>258 ± 34 (1.54)*</td>
<td>170 ± 34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>359 ± 61 (2.15)**</td>
<td>138 ± 14</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>189 ± 5</td>
<td>112 ± 13</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>202 ± 10</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>Control</td>
<td>108 ± 3</td>
<td>126 ± 4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>377 ± 142 (3.49)*</td>
<td>92.3 ± 9.6*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>840 ± 166 (7.78)**</td>
<td>67.8 ± 6.8***</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>111 ± 8</td>
<td>54.4 ± 5.7***</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>123 ± 9</td>
<td>63.6 ± 13.6***</td>
</tr>
<tr>
<td>Heart</td>
<td>Control</td>
<td>270 ± 8</td>
<td>71.1 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>362 ± 52 (1.34)*</td>
<td>67.0 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>349 ± 18 (1.29)*</td>
<td>61.5 ± 2.3*</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>245 ± 25</td>
<td>57.2 ± 1.3**</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>253 ± 9</td>
<td>58.6 ± 3.6***</td>
</tr>
<tr>
<td>Colon</td>
<td>Control</td>
<td>152 ± 4</td>
<td>115 ± 9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>294 ± 28 (1.93)**</td>
<td>119 ± 5</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>370 ± 57 (2.43)**</td>
<td>120 ± 12</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>191 ± 30</td>
<td>106 ± 7</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>170 ± 8</td>
<td>117 ± 5</td>
</tr>
</tbody>
</table>

Asterisks show a significant difference from the control values: * P < 0.05; ** P < 0.01; *** P < 0.001.
in the $K_d$ values for $^3$H]NMS binding in the submaxillary gland, heart, and colon, whereas the bladder showed a maximal enhancement in the $K_d$ values already at 1-patch/body application of oxybutynin (Table 3). The $B_{max}$ value at 2 patches/body was significantly increased in the bladder and significantly decreased in the submaxillary gland.

Effects of Intravenous Injection of Oxybutynin and DEOB on Muscarinic Receptors in Rat Tissues. At 1 h after i.v. injection of oxybutynin (7.61 μmol/kg), there was a significant increase in the $K_d$ values for specific $^3$H]NMS binding in the bladder, submaxillary gland, heart, and colon compared with the corresponding control values (Table 4). A similar degree of the enhancement in the $K_d$ values was also seen by i.v. injection of a comparable dose (8.20 μmol/kg) of DEOB in rat tissues, with the exception of the submaxillary gland that showed a significantly ($P < 0.05$) greater (17.7-fold) increase than the case (13.6-fold) by the oxybutynin injection. Both oxybutynin and DEOB produced the greatest increase in the $K_d$ values in the submaxillary gland among rat tissues. The i.v. injection of oxybutynin had little significant effect on the $B_{max}$ values for specific $^3$H]NMS binding in each tissue, whereas the injection of DEOB reduced (34.2%) the $B_{max}$ values significantly in the submaxillary gland but not in other tissues.

Concentrations of Oxybutynin and DEOB in Rat Plasma. The plasma concentrations of oxybutynin and DEOB after oral administration of oxybutynin (127 μmol/kg) reached the maximal levels (oxybutynin, 100 ± 44 nM; DEOB, 107 ± 46 nM, n = 8) at 1 h, and then they declined rapidly (Fig. 2A). The plasma concentrations of oxybutynin at
Transdermal application of oxybutynin at 0.5, 1, and 2 patches/body for 24 h in rats showed a dose-dependent increase in the plasma concentration of oxybutynin as shown by the values of 59.4 ± 7.5, 169 ± 18, and 370 ± 50 nM (n = 9–10), respectively (Fig. 3). DEOB was not detectable in the plasma of rats after transdermal application of oxybutynin at 0.5, 1, and 2 patches/body.

The plasma concentration of oxybutynin at 1 h after i.v. injection of this drug (7.61 μmol/kg) in rats was 280 ± 64 nM (n = 3), and that of DEOB at 1 h after i.v. injection of this metabolite (8.20 μmol/kg) was 505 ± 46 nM (n = 3). In the plasma of rats injected with oxybutynin, DEOB was very little detected.

**Effects of Oral and Transdermal Administration of Oxybutynin on Rat Salivary Secretion.** To examine the time-dependent change in salivation, we measured whole saliva secreted in the oral cavity of rats at 10-min intervals until 60 min after stimulation due to i.v. injection of pilocarpine (4.09 μmol/kg). The whole saliva of control rats reached a maximal level for first 10 min after the stimulation of pilocarpine, as the weights of whole saliva secreted for each 10 min after the pilocarpine injection were 688 ± 376, 156 ± 31, 370 ± 97.5, 20.3, and 46.7 ± 8.4 mg.

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Plasma concentration-time profiles of oxybutynin (●) and DEOB (▲) after oral (A) and transdermal (B) administration of oxybutynin in rats. Rats received oxybutynin orally (127 μmol/kg) or transdermally (1 patch, 33.6 μmol/body) and were then sacrificed. Blood samples were taken from the descending aorta of each rat. Each point represents the mean ± S.E. of seven to nine (oral oxybutynin) and nine (transdermal oxybutynin) rats.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Drug</th>
<th>$K_d$ (μM)</th>
<th>$B_{max}$ (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>Control</td>
<td>176 ± 5</td>
<td>154 ± 16</td>
</tr>
<tr>
<td></td>
<td>Oxybutynin</td>
<td>452 ± 83 (2.57)**</td>
<td>140 ± 19</td>
</tr>
<tr>
<td></td>
<td>DEOB</td>
<td>390 ± 45 (2.22)**</td>
<td>114 ± 13</td>
</tr>
<tr>
<td>Submaxillary gland</td>
<td>Control</td>
<td>96.3 ± 3.6</td>
<td>108 ± 8</td>
</tr>
<tr>
<td></td>
<td>Oxybutynin</td>
<td>1309 ± 136 (13.6)***</td>
<td>84.3 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>DEOB</td>
<td>1704 ± 96 (17.7)***</td>
<td>71.1 ± 4.0**</td>
</tr>
<tr>
<td>Heart</td>
<td>Control</td>
<td>230 ± 13</td>
<td>60.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>Oxybutynin</td>
<td>485 ± 43 (2.03)***</td>
<td>48.2 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>DEOB</td>
<td>451 ± 31 (1.96)***</td>
<td>52.1 ± 4.2</td>
</tr>
<tr>
<td>Colon</td>
<td>Control</td>
<td>171 ± 19</td>
<td>96.7 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>Oxybutynin</td>
<td>420 ± 10 (2.46)***</td>
<td>86.9 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>DEOB</td>
<td>442 ± 22 (2.58)***</td>
<td>91.4 ± 7.8</td>
</tr>
</tbody>
</table>

Asterisks show a significant difference from the control values: **, $P < 0.01$; ***, $P < 0.001$.
of 139 mg, respectively. As shown in Fig. 4, the weights of whole saliva secreted by the first 10 min of pilocarpine stimulation in control rats showed little significant difference, even by the repetitive agonist stimulation at 1 to 24 h.

At 1 to 24 h after oral administration of oxybutynin (127 μmol/kg), there was a marked decrease in the whole saliva compared with the value of control rats (Fig. 4A). In particular, the whole saliva at 1 and 3 h were at extremely low levels (6.8 ± 6.8 and 0.8 ± 0.8 mg, respectively, n = 5), and the pilocarpine-stimulated salivation even after 12 and 24 h was markedly attenuated as shown by the respective values of 139 ± 27 and 155 ± 37 mg. As shown in Fig. 4B, transdermal application of oxybutynin at 1 patch (33.6 μmol/body for 4, 12, and 24 h decreased the pilocarpine-stimulated salivation significantly; however, the respective weights (154 ± 88, 124 ± 29, and 146 ± 79 mg, respectively, n = 4) of whole saliva secreted in these rats were significantly (P < 0.05) larger than the values at 1 and 3 h after oral oxybutynin (Fig. 4A).

Figure 5 shows the recovery of pilocarpine-stimulated salivation in rats after oral and transdermal administration of oxybutynin. The pilocarpine-stimulated whole saliva (487 ± 43 mg, n = 4) after the 11-h removal of patch following 12-h transdermal application of oxybutynin was significantly (P < 0.001) larger than the whole saliva (139 ± 27 mg, n = 5) stimulated by pilocarpine at 12 h after oral administration of oxybutynin. Furthermore, the effects of oral and transdermal oxybutynin on the dose-response curves for pilocarpine-stimulated secretion of whole saliva were examined. The pilocarpine-stimulated secretion of whole saliva was measured 3 h after oral oxybutynin (127 μmol/kg) and after 12-h application of transdermal oxybutynin (33.6 μmol) when shown maximal enhancement of Kᵩ in the submaxillary gland (Tables 1 and 2). Oral administration of oxybutynin largely shifted dose-response curves for pilocarpine-stimulated (0.4–123 μmol/kg) salivation to the right with the concomitant reduction (65.5%) of maximal response, whereas transdermal oxybutynin showed only rightward shift of the curves (Fig. 6). The extent of shift was much smaller in the transdermal oxybutynin compared with the oral oxybutynin. The salivary secretion by each dose of pilocarpine was significantly greater in the transdermal oxybutynin-administered rats than in the oral oxybutynin-administered rats.

**Discussion**

A number of previous studies have substantiated the usefulness of ex vivo and in vivo receptor binding assay to clarify the potency, organ selectivity, and duration of action of drugs in relation to their pharmacokinetics and pharmacodynamics (Beauchamp et al., 1995; Uchida et al., 1995; Ohkura et al., 1998; Yamada et al., 1998, 1999, 2001). In the present study, ex vivo muscarinic receptor binding of transdermal oxybuty-
Transdermal application of oxybutynin at 0.5, 1, and 2
receptors in rat tissues at a slow and reversible manner.
appeared by the removal of patch. These data suggest that
maxillary gland after transdermal oxybutynin promptly dis-
binding in these tissues. Given that an increase in
rat tissues. Thus, oral but not trans-
dermal oxybutynin produced extremely long-lasting reduc-
tion of muscarinic receptor sites in the submaxillary gland
heart, suggesting noncompetitive blockade of musca-
rinic receptors due to oral oxybutynin. Such antagonism
might be due to slowly dissociating blockade by oral oxy-
butynin of muscarinic receptors in these tissues, as dem-
onstrated previously by in vitro and in vivo receptor bind-
ing studies (Yamada et al., 1985, 1999).

The observed difference between oral and transdermal oxy-
butynin in ex vivo muscarinic receptor binding characteris-
tics to the submaxillary gland has been further realized by
the mode of antagonism against cholinergic salivation. The
pilocarpine-stimulated salivation in rats was completely
abolished 1 and 3 h after oral oxybutynin, followed by the
marked and sustained suppression of salivation. In contrast,
transdermal application of oxybutynin for 2 to 24 h sup-
pressed significantly pilocarpine-stimulated salivation but it
did not abolish secretory response. In other words, rapid
cessation of salivation induced by oral oxybutynin did not
occur in rats receiving transdermal oxybutynin and further
pilocarpine-induced salivation was promptly recovered by
the removal of patch (Fig. 5). In addition, in dose-response
curves of pilocarpine-induced salivation, the antagonism by
oral oxybutynin, unlike transdermal oxybutynin, was not
simply competitive in that it suppressed markedly maximal
response by pilocarpine. Such insurmountable antagonism
agrees well with a sustained decrease of $[^3]$H]NMS binding
sites ($B_{\text{max}}$) in the submaxillary gland after oral but not
transdermal oxybutynin. Thus, it is worth noting that the
mode of inhibition of pilocarpine-stimulated salivation after oral and transdermal oxybutynin are in ac-
 accord with those of exocrine muscarinic receptor binding.

Based on the magnitude of increases in $K_d$ values, musca-
rinic receptor binding activity of both oral and transdermal
oxybutynin was greatest in the submaxillary gland, followed
by the bladder, colon, and heart. Our recent data with $M_1$
and $M_2$ subtype knockout mice have shown that $M_3$
subtype is expressed predominantly in the submaxillary gland
and moderately in the prostate and bladder, whereas $M_2$
subtype is major subtype present in the bladder, heart, and colon
(T. Oki, et al., unpublished observation). Because oxybutynin

exerted higher affinity to M₃ subtype (Nilvebrant and Sparf, 1982; Nilvebrant et al., 1997; Oki et al., 2005), the greater receptor binding activity of this drug in the submaxillary gland may therefore reflect M₃ subtype selectivity.

It is known that oxybutynin is rapidly absorbed from gastrointestinal tract and that its major pathway of elimination is hepatic metabolism (Douchamps et al., 1988) and that plasma concentration of DEOB in human after oral administration is much higher than that of oxybutynin (Hughes et al., 1992). In fact, DEOB, as well as oxybutynin, exhibits potent relaxation of human detrusor smooth muscles mainly through the blockade of muscarinic receptors (Waldeck et al., 1997). Therefore, it is considered that DEOB makes significant contribution to the pharmacological effect of oxybutynin. In the present study, similar concentrations of oxybutynin and DEOB were detected in the plasma of rats after oral oxybutynin, reaching a maximal level at 1 h followed by a rapid decline. The plasma concentration of transdermal oxybutynin in rats increased time dependently until 12 h of application and dose dependent at 0.5 to 2 patches/body. On the other hand, little DEOB was detected in the plasma of rats receiving transdermal oxybutynin. These data indicate that transdermal application of oxybutynin produces a slow increase in the plasma concentration of oxybutynin and that this route of administration of oxybutynin efficiently avoids first-pass effect as reported in healthy subjects (Appell et al., 2003; Zobrist et al., 2003).

Based on previous reports that muscarinic receptor binding affinity of DEOB was greater in the salivary gland than in the bladder (Waldeck et al., 1997; Oki et al., 2005; Maruyama et al., 2006), it is considered that DEOB may be responsible for long-lasting occupation of exocrine muscarinic receptors after oral oxybutynin. In fact, significantly greater enhancement by DEOB of Kᵦ values for specific [³H]NMES binding in the rat submaxillary gland was seen after the i.v. injection of approximately equimolar doses of oxybutynin and DEOB. Furthermore, significant decrease of Bₘₐₓ values in the submaxillary gland was observed only by the injection of DEOB. These data suggest that DEOB occupies muscarinic receptors in the exocrine gland with a greater extent and for a longer period than oxybutynin. As described above, similar concentration of DEOB as oxybutynin was detected 1 and 3 h after oral oxybutynin in rats, but little of this metabolite was detected after transdermal oxybutynin. Taken together, these data suggest that DEOB contributes, at least in part, to the long-lasting occupation of muscarinic receptors in the exocrine gland. Such an idea is consistent with clinical observation that lower plasma DEOB during oxybutynin treatment may be closely associated with greater saliva output in healthy subjects (Sathyan et al., 2001; Appell et al., 2003).

In addition, it is also assumed that a steep increase in the plasma concentration of oxybutynin and DEOB after oral oxybutynin causes insurmountable antagonism against muscarinic receptors and cholinergic salivation by exposing these exocrine receptors to extremely high concentrations of both agents. Consequently, it is possible that pharmacokinetic characteristics, such as substantially lower fluctuation in plasma oxybutynin and avoidance of first-pass effect by transdermal oxybutynin, brings about significant difference of exocrine muscarinic receptor binding characteristics from that in oral oxybutynin, leading to the lower incidence of dry mouth.

In conclusion, the present study has shown that transdermal oxybutynin leads to a significant degree of binding to rat bladder muscarinic receptors without causing both long-lasting occupation of muscarinic receptors in the submaxillary gland and abolishment of salivation evoked by oral oxybutynin. Thus, the present study provides further pharmacological evidence for advantage of transdermal over oral oxybutynin in the therapy of overactive bladder.

Acknowledgments

We thank Akihiro Kawashima and Dr. Masayuki Uchida (Meiji Milk Products Co. Ltd., Odawara, Japan) for providing valuable information.

References


### Address correspondence to:

Dr. Shizuo Yamada, Department of Pharmacokinetics and Pharmacodynamics and Center of Excellence Program in the 21st Century, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8528, Japan. E-mail: yamada@ys7.u-shizuoka-ken.ac.jp