Muscarinic Receptor Binding Characteristics in Rat Tissues after Oral Administration of Oxybutynin and Propiverine

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Ex vivo muscarinic receptor binding of oxybutynin and propiverine, the most commonly used anticholinergic agents for the treatment in patients with urinary incontinence, was investigated in rat tissues. The oral administration of oxybutynin (50.8 and 127 $\mu\text{mol/kg}$) caused a significant increase in the apparent dissociation constant (K_d) for specific (–)-[³H]QNB binding in the rat bladder, prostate, submaxillary gland, heart and cerebral cortex, compared with each of the control values. Also, in the submaxillary gland of these rats, there was a reduction in the maximal number of binding sites (B_{max}) for (–)-[³H]QNB binding. Similarly, oral administration of propiverine at doses of 74.3–297 $\mu\text{mol/kg}$ brought about a significant increase in the K_d values for (–)-[³H]QNB binding in rat tissues including the bladder, and greater increase in K_d values was seen in the rat prostate, heart and submaxillary gland. On the other hand, oral administration of propiverine, unlike oxybutynin, resulted in very little reduction in the B_{max} values for (–)-[³H]QNB binding in the submaxillary gland. In conclusion, the present study has shown that oxybutynin and propiverine, after oral administration, bind significantly to muscarinic receptors in tissues such as the bladder, which is the target organ for the treatment of urinary incontinence, and that oxybutynin appears to exhibit long-term binding to muscarinic receptors in the salivary gland.

Key words oxybutynin; propiverine; bladder; submaxillary gland; muscarinic receptor binding

Urinary incontinence and other symptoms of lower urinary tract dysfunction are very common in the rapidly growing geriatric population. Urge urinary incontinence with bladder instability (detrusor instability or detrusor hyperreflexia) is among the most common type of incontinence in geriatric patients.¹⁾ Anticholinergic drugs, such as oxybutynin, are widely used to treat this symptom since parasympathetic innervation is the predominant stimulus for bladder contraction.²⁾ However, anticholinergic drugs can cause problems in geriatric patients because of their side effects, such as dry mouth, blurred vision, constipation and tachycardia.³⁾ The majority of both the beneficial and unwanted effects of these drugs in patients with bladder instability stems from the blockade of muscarinic receptors in target (bladder) and non-target tissues. In an attempt to reduce serious side-effects, such as dry mouth, a number of compounds have been synthesized and evaluated in terms of pharmacological specificity in the bladder relative to the parotid gland.^{4,5)} Also, a new drug delivery system for oxybutynin has been used clinically and found to be an effective dosage form.⁶⁾

Although there are many studies dealing with the pharmacological effects and *in vitro* receptor binding affinities of anticholinergic agents,^{3,7–9)} very little *in vivo* data are available regarding the muscarinic receptor binding characteristics (extent and time-course) in tissues following systemic administration of anticholinergic agents. In general, the results of *in vitro* receptor binding studies of drugs may not necessarily guarantee *in vivo* pharmacological specificity, such as organ selectivity, because they do not take into account various pharmacokinetic (absorption, distribution and elimination) and pharmacodynamic factors.¹⁰⁾ This was the case for the receptor binding properties of the 1,4-dihydropyridine calcium channel antagonists^{11,12)} and α_1 -adrenoceptor antagonists.^{13–15)} Thus, the aim of the present study was to characterize the muscarinic receptor binding in rat tissues, such as the bladder, after oral administration of oxybutynin and

propiverine, the most commonly used anticholinergic agents for the treatment of patients with detrusor instability and detrusor hyperreflexia.

MATERIALS AND METHODS

Materials (–)-[*N*-methyl-³H]quinuclidinylbenzylate methylchloride ((–)-[³H]QNB, 3.09 TBq/mmol) was purchased from Dupont-NEN Co., Ltd. (Boston, MA, U.S.A.). Oxybutynin hydrochloride (oxybutynin) and propiverine hydrochloride (propiverine) were generously donated by Meiji Milk Products Co., Ltd. (Odawara, Japan) and Taiho Pharmaceutical Co., Ltd. (Saitama, Japan), respectively. All other chemicals were purchased from commercial sources.

Tissue Preparation Male Sprague Dawley rats weighing about 200–300 g were housed, three to four per cage, in the laboratory with free access to food and water, and were maintained on a 12 h dark/light cycle in a room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$). They were fasted for 16 h before drug administration, then given propiverine (24.8–297 $\mu\text{mol/kg}$) or oxybutynin (50.8, 127 $\mu\text{mol/kg}$) orally in the form of a suspension in distilled water. Control animals were given vehicle alone. At 0.5 to 6 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. Then, the bladder, prostate, submaxillary gland, heart and cerebral cortex were dissected, and fat and blood vessels removed by trimming. The tissues were minced with scissors and homogenized by a Kinematica Polytron homogenizer in 20 volumes of ice-cold 50 mM Na^+/K^+ phosphate buffer (pH 7.4). The homogenates were centrifuged at $40000 \times g$ for 20 min and the resulting pellets finally suspended in buffer for the binding assay. In the *ex vivo* experiment, there is a possibility that oxybutynin and propiverine might partially dissociate from the receptor sites

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during tissue preparation (homogenization and suspension) after oral administration to rats. Yamada *et al.*¹⁶ previously showed that the dissociation of antagonists from receptor sites at 4 °C was extremely slow. So, to minimize the dissociation of oxybutynin and propiverine from the receptor sites, all steps involved in the tissue preparation were performed at 4 °C. In fact, there was no large difference in (–)-[³H]QNB binding parameters (K_d and B_{max}) between rat bladder homogenates washed once or twice at 0.5 h after oral administration of oxybutynin (127 μ mol/kg) or at 1 h after the administration of propiverine (149 μ mol/kg). Protein concentrations were measured by the method of Lowry *et al.*¹⁷

(–)-[³H]QNB Binding Assay The binding assay for muscarinic receptors was performed using (–)-[³H]QNB as previously described.^{18,19} The homogenates (90–600 μ g of protein) of rat tissues were incubated with different concentrations (0.06–1.5 nM) of (–)-[³H]QNB in 50 mM of a Na⁺/K⁺ phosphate buffer (pH 7.4). Incubation was carried out for 60 min at 37 °C. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD, U.S.A.) through Whatman GF/B glass fiber filters, and the filters were 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 2,5-diphenyloxazole, 0.3 g 1,4-bis[2-(5-phenyloxazolyl)]benzene), and the radioactivity was determined in a liquid scintillation counter. Specific (–)-[³H]QNB binding was determined experimentally from the difference between counts in the absence and presence of 1 μ M atropine. All assays were conducted in duplicate.

Data Analysis The analysis of (–)-[³H]QNB binding data was performed as described previously.¹⁶ The apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for (–)-[³H]QNB were estimated by Rosenthal analysis of the saturation data. Statistical analysis of the data was performed by a one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparison. Statistical significance was accepted at $p < 0.05$.

RESULTS

Effects of Oral Administration of Oxybutynin The effect of oral administration of oxybutynin on specific (–)-[³H]QNB binding in the rat bladder, prostate, submaxillary gland and heart was investigated. Three hours after oral administration of oxybutynin, at a dose of 50.8 μ mol/kg, there was a significant (57%) increase in K_d values for specific (–)-[³H]QNB binding in the rat bladder compared with the control value (Table 1). In the prostate and submaxillary gland of these rats, the K_d values were also significantly increased at 0.5 and 3 h, and the magnitude of this increase was 99–101% (prostate) and 131–138% (submaxillary gland). Furthermore, the significant increases in the K_d values in the heart after 3 and 6 h were 75 and 20%, respectively. The B_{max} value for specific (–)-[³H]QNB binding in each tissue remained largely unchanged except for a significant (20–36%) reduction in the submaxillary gland at 0.5–6 h after oxybutynin administration.

The higher oral dose (127 μ mol/kg) of oxybutynin caused a sustained increase in the K_d values for (–)-[³H]QNB binding in each tissue at 0.5, 3 and 6 h, and the magnitude of this

Table 1. K_d and B_{max} for Specific (–)-[³H]QNB Binding in the Bladder, Prostate, Submaxillary Gland, Heart and Cerebral Cortex of Rats, 0.5 to 6 h after Oral Administration (*p.o.*) of Oxybutynin (50.8 μ mol/kg)

Organ	Time after <i>p.o.</i> (h)	K_d (pM)	B_{max} (fmol/mg protein)
Bladder	Control	130 ± 10	175 ± 12
	0.5	166 ± 20	150 ± 24
	3	204 ± 37**	165 ± 34
	6	130 ± 21	163 ± 14
Prostate	Control	213 ± 20	95.9 ± 5.54
	0.5	429 ± 159**	80.6 ± 18.9
	3	423 ± 132**	83.0 ± 22.7
	6	261 ± 41	81.0 ± 7.19
Submaxillary gland	Control	257 ± 21	127 ± 3.62
	0.5	594 ± 302**	102 ± 17**
	3	612 ± 194***	93.7 ± 10.5***
	6	300 ± 32	81.0 ± 12.4***
Heart	Control	207 ± 9.7	69.3 ± 4.71
	0.5	281 ± 132	70.8 ± 4.3
	3	362 ± 163*	73.6 ± 5.1
	6	249 ± 17*	66.9 ± 6.6
Cerebral cortex	Control	151 ± 6	698 ± 23
	0.5	289 ± 67**	715 ± 25
	3	175 ± 9	711 ± 15
	6	160 ± 4	689 ± 10

Values are mean ± S.E. of 4 to 10 rats. Rats received 50.8 μ mol/kg of oxybutynin orally, and were sacrificed 0.5 to 6 h later. Specific binding of (–)-[³H]QNB (0.06–1.5 nM) in rat tissues was measured. Asterisks show a significant difference from the control values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 2. K_d and B_{max} for Specific (–)-[³H]QNB Binding in the Bladder, Prostate, Submaxillary Gland, Heart and Cerebral Cortex of Rats, 0.5 to 6 h after Oral Administration (*p.o.*) of Oxybutynin (127 μ mol/kg)

Organ	Time after <i>p.o.</i> (h)	K_d (pM)	B_{max} (fmol/mg protein)
Bladder	Control	130 ± 10	175 ± 12
	0.5	221 ± 24**	153 ± 8.7
	3	243 ± 22***	155 ± 8.3
	6	194 ± 18**	139 ± 5.9*
Prostate	Control	213 ± 20	95.9 ± 5.54
	0.5	549 ± 123**	94.1 ± 2.92
	3	854 ± 243**	75.9 ± 7.85
	6	443 ± 32***	68.2 ± 6.49**
Submaxillary gland	Control	257 ± 21	127 ± 3.62
	0.5	823 ± 151***	114 ± 7.94
	3	1427 ± 273***	88.0 ± 7.08***
	6	676 ± 61***	80.3 ± 3.09***
Heart	Control	207 ± 9.7	69.3 ± 4.71
	0.5	296 ± 20.5**	73.0 ± 2.94
	3	329 ± 14.4***	76.9 ± 1.69
	6	284 ± 8.03***	71.3 ± 3.16
Cerebral cortex	Control	151 ± 6	698 ± 23
	0.5	253 ± 36	699 ± 21
	3	321 ± 76**	698 ± 9
	6	197 ± 15	645 ± 13

Values are mean ± S.E. of 2 to 10 rats. Rats received 127 μ mol/kg of oxybutynin orally, and were sacrificed 0.5 to 6 h later. Specific binding of (–)-[³H]QNB (0.06–1.5 nM) in rat tissues was measured. Asterisks show a significant difference from the control values, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

increase was 49–87% (bladder), 108–301% (prostate), 163–455% (submaxillary gland) and 37–59% (heart), respectively (Table 2). This enhancement, compared with that produced by 50.8 μ mol/kg of this drug (Table 1), was greater in the bladder, prostate and submaxillary gland, but comparable in the heart. In rats given oxybutynin (127 μ mol/kg), the B_{max} values were significantly reduced at 6 h in the bladder

Table 3. K_d and B_{max} for Specific (–)-[³H]QNB Binding in the Bladder, Prostate, Submaxillary Gland, Heart and Cerebral Cortex of Rats, 1 h after Oral Administration (*p.o.*) of Propiverine (24.8–297 μ mol/kg)

Organ	Doses (μ mol/kg)	K_d (pM)	B_{max} (fmol/mg protein)
Bladder	Control	147 $\pm$ 13	167 $\pm$ 16
	24.8	170 $\pm$ 21	132 $\pm$ 13
	74.3	192 $\pm$ 26	115 $\pm$ 7*
	149	198 $\pm$ 12*	138 $\pm$ 12
	297	197 $\pm$ 12*	132 $\pm$ 18
Prostate	Control	168 $\pm$ 9	64.9 $\pm$ 7.0
	24.8	208 $\pm$ 24	58.2 $\pm$ 10.8
	74.3	318 $\pm$ 31***	59.2 $\pm$ 5.3
	149	342 $\pm$ 32***	61.2 $\pm$ 7.4
	297	278 $\pm$ 50*	45.5 $\pm$ 13.5
Submaxillary gland	Control	243 $\pm$ 17	124 $\pm$ 7
	24.8	314 $\pm$ 6*	109 $\pm$ 7
	74.3	523 $\pm$ 84**	103 $\pm$ 11
	149	513 $\pm$ 91**	103 $\pm$ 4
	297	401 $\pm$ 19**	98.3 $\pm$ 8.4
Heart	Control	209 $\pm$ 11	75.2 $\pm$ 2.9
	24.8	246 $\pm$ 25	74.6 $\pm$ 4.1
	74.3	399 $\pm$ 61**	85.4 $\pm$ 5.9
	149	359 $\pm$ 39**	77.8 $\pm$ 4.7
	297	341 $\pm$ 35**	77.7 $\pm$ 1.4
Cerebral cortex	Control	161 $\pm$ 4	737 $\pm$ 16
	24.8	189 $\pm$ 13	851 $\pm$ 13
	74.3	277 $\pm$ 40**	770 $\pm$ 27
	149	222 $\pm$ 6***	713 $\pm$ 54
	297	249 $\pm$ 17***	835 $\pm$ 30

Values are mean $\pm$ S.E. of 3 to 6 rats. Rats received 24.8 to 297 μ mol/kg of propiverine orally, and were sacrificed 1 h later. Specific binding of (–)-[³H]QNB (0.06–1.0 nM) in rat tissues was measured. Asterisks show a significant difference from the control values, * p <0.05, ** p <0.01, *** p <0.001.

and prostate, and at 3 and 6 h in the submaxillary gland.

One-half and 3 h after oral administration of oxybutynin (50.8 and 127 μ mol/kg), the K_d values for cerebral cortical (–)-[³H]QNB binding were increased by 68–113% (Tables 1 and 2).

Effects of Oral Administration of Propiverine The effect of oral administration of propiverine on specific (–)-[³H]QNB binding in the rat bladder, prostate, submaxillary gland and heart was investigated. One hour after oral administration of propiverine at doses of 74.3, 149 and 297 μ mol/kg, there was an increase (31, 35 and 34%, respectively) in the K_d values for specific (–)-[³H]QNB binding in the rat bladder compared with the control value, and the enhancement at 149 and 297 μ mol/kg was statistically significant (Table 3). Administration of 74.3 μ mol/kg propiverine caused a significant (31%) reduction in the B_{max} value for (–)-[³H]QNB binding in the bladder. Similarly, the K_d values for (–)-[³H]QNB in the prostate, heart and cerebral cortex were significantly (38–104%) increased by oral administration of propiverine at doses of 74.3, 149 and 297 μ mol/kg. In the submaxillary gland, oral administration of propiverine at the doses of 24.8, 74.3, 149 and 297 μ mol/kg resulted in a significant increase (29, 115, 111 and 65%, respectively) in the K_d values for (–)-[³H]QNB binding. Thus, the increase in K_d values for (–)-[³H]QNB binding in each tissue appeared to be maximal at an oral dose of 74.3 μ mol/kg propiverine, and there was little more/additional increase in K_d values following administration of higher doses of propiverine. On the other hand, there were few significant al-

Table 4. K_d and B_{max} for Specific (–)-[³H]QNB Binding in the Bladder, Prostate, Submaxillary Gland, Heart and Cerebral Cortex of Rats, 0.5 to 6 h after Oral Administration (*p.o.*) of Propiverine (74.3 μ mol/kg)

Organ	Time after <i>p.o.</i> (h)	K_d (pM)	B_{max} (fmol/mg protein)
Bladder	Control	147 $\pm$ 13	167 $\pm$ 16
	0.5	175 $\pm$ 13	125 $\pm$ 1
	1	192 $\pm$ 26	115 $\pm$ 7*
	3	221 $\pm$ 30*	147 $\pm$ 18
	6	174 $\pm$ 10	124 $\pm$ 13
	Control	168 $\pm$ 9	64.9 $\pm$ 7.0
Prostate	0.5	291 $\pm$ 45**	57.9 $\pm$ 9.7
	1	318 $\pm$ 31***	59.2 $\pm$ 5.3
	3	323 $\pm$ 28***	77.9 $\pm$ 9.7
	6	245 $\pm$ 29*	57.8 $\pm$ 10.5
	Control	243 $\pm$ 17	124 $\pm$ 7
	0.5	438 $\pm$ 48**	128 $\pm$ 11
Submaxillary gland	1	523 $\pm$ 84**	103 $\pm$ 11
	3	521 $\pm$ 59***	111 $\pm$ 4
	6	374 $\pm$ 24**	125 $\pm$ 8
	Control	209 $\pm$ 11	75.2 $\pm$ 2.9
	0.5	292 $\pm$ 10**	91.8 $\pm$ 5.2*
	1	399 $\pm$ 61**	85.4 $\pm$ 5.9
Heart	3	340 $\pm$ 18***	105 $\pm$ 9**
	6	252 $\pm$ 31	66.2 $\pm$ 4.3
	Control	161 $\pm$ 4	737 $\pm$ 16
	0.5	199 $\pm$ 15*	764 $\pm$ 29
	1	277 $\pm$ 40**	770 $\pm$ 27
	3	266 $\pm$ 18***	783 $\pm$ 9
Cerebral cortex	6	239 $\pm$ 18**	796 $\pm$ 63

Values are mean $\pm$ S.E. of 3 to 6 rats. Rats received 74.3 μ mol/kg of propiverine orally, and were sacrificed 0.5 to 6 h later. Specific binding of (–)-[³H]QNB (0.06–1.0 nM) in rat tissues was measured. Asterisks show a significant difference from the control values, * p <0.05, ** p <0.01, *** p <0.001.

terations in B_{max} values for (–)-[³H]QNB binding in the prostate, submaxillary gland or heart.

The time-course of the effect of propiverine on specific (–)-[³H]QNB binding in each tissue was examined. Following oral administration of propiverine (74.3 μ mol/kg), there was an increase in the K_d value for (–)-[³H]QNB binding in the bladder, a significant reduction in the B_{max} at 1 h, and significant (50%) increase in the K_d at 3 h (Table 4). There were constant increases (46–92, 54–115 and 24–72%, respectively) in the K_d values in the prostate, submaxillary gland and cerebral cortex 0.5–6 h after propiverine administration, and also an increase (40–91%) in the heart 0.5–3 h. In the heart, there was a significant increase in the B_{max} at 0.5 and 3 h.

DISCUSSION

To date, little information has been published regarding the muscarinic receptor binding characteristics in tissues after oral administration of oxybutynin and propiverine. The technique of *ex vivo* and *in vivo* receptor binding is useful for predicting the *in vivo* potency, organ selectivity, and duration of action of drugs in relation to their pharmacokinetics and pharmacodynamics.^{10–15} In the present study, the *ex vivo* receptor binding technique was used to simultaneously examine muscarinic receptor binding characteristics in the bladder and other tissues of rats after systemic administration of oxybutynin and propiverine. The oral doses of oxybutynin and propiverine chosen were 50.8 or 127 μ mol/kg and 24.8–

297 $\mu\text{mol/kg}$, respectively, because they were expected to produce significant pharmacological effects on the basis of intravenous doses of these drugs in reducing urinary bladder contractions in rat cystometry studies.^{20–22)}

The oral administration of oxybutynin (50.8 and 127 $\mu\text{mol/kg}$) produced a significant increase in the K_d values for (–)-[³H]QNB binding in the bladder, prostate, submaxillary gland and heart of rats, compared with the corresponding control value. The increase in K_d values was seen at 0.5, 3 and 6 h in all tissues after administration of a higher dose of drug, and the effect was greatest in the submaxillary gland, followed by the prostate, heart and bladder. The observed enhancement by oxybutynin in the K_d values for (–)-[³H]QNB binding suggests that this drug binds competitively to muscarinic receptors in rat tissues after oral administration. On the other hand, a higher dose of oxybutynin produced a significant reduction in the B_{max} of (–)-[³H]QNB binding in the bladder and prostate but only at 6 h. Interestingly, there was a constant and sustained reduction in the B_{max} values for (–)-[³H]QNB binding only in the rat submaxillary gland following both doses of oxybutynin, suggesting a competitive and noncompetitive blockade of muscarinic receptors. Such antagonism is believed to be due to the slowly dissociating blockade by oxybutynin of muscarinic receptors in the rat submaxillary gland, as demonstrated previously in the non-equilibrium blockade of brain nicotinic receptors by neosurugatoxin.²³⁾ It is speculated that such receptor binding specificity of oxybutynin in the exocrine gland may account, at least in part, for the high incidence of severe dry mouth after oral administration of this drug in patients with urinary incontinence.³⁾

The oral administration of propiverine at doses of 74.3–297 $\mu\text{mol/kg}$ produced a considerable increase in the K_d values for (–)-[³H]QNB binding with a significant reduction in the B_{max} (only at the dose of 74.3 $\mu\text{mol/kg}$) in the rat bladder. Also, a greater increase in the K_d values was seen in the rat prostate, heart and submaxillary gland following propiverine administration. Such an increase in each tissue seems to reach a plateau at the dose of 74.3 $\mu\text{mol/kg}$ for this drug. From the time-course of the K_d values for (–)-[³H]QNB binding after propiverine administration, it appears that propiverine may have a more sustained inhibitory effect on the muscarinic receptors in the prostate and submaxillary gland than in the bladder and heart. However, oral administration of propiverine, unlike oxybutynin, produced very little reduction in the B_{max} values for (–)-[³H]QNB binding in the submaxillary gland. This observation suggests a more reversible binding of propiverine than oxybutynin to the muscarinic receptors in the salivary gland. In connection with this observation, it is of some interest to note that propiverine has less effect on salivation than oxybutynin in conscious dogs,²⁴⁾ and that propiverine may be better tolerated than oxybutynin, particularly with respect to the frequency and intensity of the episodes of dry mouth in patients with urinary incontinence.²⁵⁾ The reason for a significant increase in the B_{max} values for (–)-[³H]QNB binding in the rat heart 0.5 and 3 h after oral administration of propiverine is not clear, but it may reflect up-regulation of myocardial muscarinic receptors as a result of the receptor blockade as previously reported in tissues of atropine-treated rats.²⁶⁾

Oral administration of oxybutynin and propiverine consid-

erably increased the K_d values for cerebral cortical (–)-[³H]QNB binding with little effect on the B_{max} values, and the effect was more marked in the brain of propiverine-treated rats. These data may indicate that both drugs could easily cross the blood-brain barrier and bind to muscarinic receptor sites in the brain parenchymal cells to a significant degree *in vivo*. Hence, it is plausible that oxybutynin and propiverine exert some CNS effects *in vivo*, due to the blockade of brain muscarinic receptors, although, to our knowledge, such anticholinergic effects of these drugs have not been identified directly. In this connection, it is of some interest to note the recent finding by Matsuo *et al.*²⁷⁾ that intraperitoneal injection of oxybutynin and propiverine induced catalepsy in mice in a dose-dependent manner and their effects might be closely associated with the *in vivo* blockade of dopamine and muscarinic receptors in the CNS.

In conclusion, this study has shown that oxybutynin and propiverine, after oral administration, exhibit a significant degree of binding to the muscarinic receptors in tissues such as the bladder, which is the target organ for the treatment of urinary incontinence, and that oxybutynin exhibits longer term occupation of muscarinic receptors in the salivary gland than propiverine. Thus, the present technique, which measures muscarinic receptor binding parameters after treatment with anticholinergic drugs under physiological conditions, may offer a better insight into the organ-selectivity of these drugs than results obtained from *in vitro* experiments.

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