Effects of Pilocarpine, a Muscarinic Receptor Agonist, on Contraction of the Pig and Human Urinary Bladder

Tomohiko Kamasako1, Kanya Kaga1, Mayuko Kaga1, Miki Fuse1, Mitsuru Ishizuka2 and Tomonori Yamanishi1*  
1Department of Urology, Continence Center, Dokkyo Medical University, Tochigi, Japan  
2Department of Surgery, Continence Center, Dokkyo Medical University, Tochigi, Japan

Abstract

Background and Objective: Cholinergic drugs have been considered for the treatment of underactive bladder; however, they have not been standardized due to lack of efficacy and serious side effects. This study examined the effects of pilocarpine, a muscarinic receptor agonist, on contraction of pig and human urinary bladder.

Materials and Methods: Strips of bladder tissues were mounted in 10-ml organ baths containing Krebs solution, which was maintained at 37°C and continuously gassed with 95% O2 and 5% CO2. Cumulative Concentration-Response Curves (CRCs) to pilocarpine were obtained, in the presence of Krebs solution containing darifenacin, 4-Diphenyl Acetoxy-Methyl Piperidine Methiodide (4-DAMP) (M1 selective antagonist), pirenzepine (M2 selective antagonist), methoctramine (M2 selective antagonist), or in the presence of vehicle.

Results: Pilocarpine induced contractions of smooth muscle of the detrusor in a concentration-dependent manner, with maximum contraction relative to 80 mM KCl of 134.4% and 78%, respectively, and pEC50 values of 5.28 and 5.1, respectively, in the pig and human bladder. Darifenacin, 4-DAMP, pirenzepine, and methoctramine caused surmountable antagonism of responses to pilocarpine, with Schild plot slopes of 1.37 ± 0.20, 0.80 ± 0.54, 1.05 ± 0.30, and 0.91 ± 0.35, respectively, in the pig bladder. The rank order of mean pA2 values was as follows: 4-DAMP (8.79 ± 0.27) = darifenacin (8.73 ± 0.06) > pirenzepine (6.72 ± 0.12) > methoctramine (6.58 ± 0.16). Darifenacin caused surmountable antagonism of responses to pilocarpine, with Schild plot slope of 0.93 ± 0.30 and a pA2 value of 8.85 ± 0.13 in the human bladder.

Conclusion: Pilocarpine appears to produce contraction of the pig and human bladder through activation of M3-muscarinic receptor.

Keywords: Pilocarpine; Muscarinic receptor; Urinary bladder; Pig; In vitro

Introduction

Contraction of urinary bladder is mediated by activation of muscarinic receptors, with M3-muscarinic receptors predominant [1]. Cholinergic drugs such as bethanechol chloride and distigmine have been considered to enhance detrusor contractility and promote bladder emptying in patients with underactive bladder. However, the use of cholinergic drugs has not been standardized, due to lack of efficacy and serious side effects.

Recently, pilocarpine, a muscarinic receptor agonist, has been reported to be effective for the treatment of dryness of eyes or salivation disorders [2-5]. However, the effects of pilocarpine on contraction of urinary bladder have not been studied.

The aim of this study was to examine the effects of pilocarpine on contraction of pig and human urinary bladder in vitro.

Materials and Methods

Pig urinary bladder was collected from an abattoir. Strips of human bladder tissues were obtained from patients who had undergone total cystectomy for bladder cancer, and the tissues were free from tumor. The procedures were approved by the local ethics committee of our institution (No. 22184), and written informed consent was obtained from each patient before enrollment.

Strips of tissues (8 mm × 2 mm) were obtained, and the serosa and urothelium were removed.

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Correspondence: Tomonori Yamanishi, Department of Urology, Continence Center, Dokkyo Medical University, 880 Kitakobayashi, Mibu, Tochigi, 321-0293, Japan, Tel: +81 282 86 1111; Fax: +81 282 86 5105; E-mail: yamanishi@dokkyomed.ac.jp

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• Correspondence: Tomonori Yamanishi, Department of Urology, Continence Center, Dokkyo Medical University, 880 Kitakobayashi, Mibu, Tochigi, 321-0293, Japan, Tel: +81 282 86 1111; Fax: +81 282 86 5105; E-mail: yamanishi@dokkyomed.ac.jp

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Muscle strips were mounted in 10-ml organ baths containing Krebs solution (composition in mM: NaCl 118.4, KCl 4.7, CaCl₂ 1.9, NaHCO₃ 24.9, MgSO₄ 1.15, KH₂PO₄ 1.15, glucose 11.7), which was maintained at 37°C and continuously gassed with 95% O₂ and 5% CO₂. The tissues were subjected to a resting tension of 1.0 g (9.8 mN) and allowed to equilibrate for 60 min, during which time they were washed every 10 min and the resting tension was adjusted. The isometric tension generated by each muscle specimen was measured using a Power Lab data acquisition system (ADInstruments Pty Ltd., Bella Vista, New South Wales, Australia).

Cumulative Concentration-Response Curves (CRCs) to pilocarpine were obtained. Each muscle strip was then washed for about 45 min until a stable resting tension was attained, followed by equilibration for 30 min with Krebs solution containing the appropriate concentration of antagonist or vehicle (time control). In the control experiments, the second CRCs to pilocarpine were not reproducible, probably because of tachyphylaxis. Each tissue was used only to construct one CRC to pilocarpine with or without one concentration of a muscarinic antagonist, and affinity values for each antagonist were calculated.

In the pig urinary bladder, darifenacin, 4-diphenyl acetoxy-methyl piperidine methiodide (4-DAMP) (M₁ selective antagonist), pirenzepine (M₁ selective antagonist), or methoctramine (M₂ selective antagonist) was used as a muscarinic receptor antagonist, and affinity values for each antagonist were calculated.

In human tissues, cumulative Concentration-Response Curves (CRCs) to pilocarpine were obtained, in the presence of Krebs solution containing darifenacin or in the presence of vehicle. Darifenacin was treated for 30 min before the addition of pilocarpine.

In the pig bladder, darifenacin, 4-DAMP, pirenzepine, and methoctramine caused surmountable antagonism of responses to pilocarpine, with Schild plot slopes of 1.37 ± 0.20, 0.80 ± 0.54, 1.05 ± 0.30, and 0.91 ± 0.35, respectively, in the pig bladder. The rank order of mean pA² values was as follows: 4-DAMP (8.79 ± 0.27) = darifenacin (8.73 ± 0.06) > pirenzepine (6.72 ± 0.12) > methoctramine (6.58 ± 0.16) (Figure 1A-1D).

In the human bladder, darifenacin caused surmountable antagonism of responses to pilocarpine, with Schild plot slope of 0.93 ± 0.30 and pA² value of 8.85 ± 0.13 in the human bladder (Figure 2).

**Discussion**

For the management of voiding difficulty in patients with an...
underactive detrusor, clean intermittent catheterization is used as the first choice of treatment. However, complications can occur in clean intermittent catheterization, and there are many patients who want to urinate by themselves even if it requires straining or the Credé maneuver, or because they reject self-catheterization due to pain, etc. Drug therapy can enable natural voiding and is ideal for increasing the patient’s quality of life, provided the risk of upper urinary tract deterioration or infection can be avoided.

Bethanechol chloride, a choline ester, acts on muscarinic receptors with only a feeble nicotinic effect, while distigmine bromide, a choline esterase inhibitor, sustains acetylcholine activity. These drugs have been considered to enhance detrusor contractility and promote bladder emptying in patients with underactive bladders. Oral administration of bethanechol and distigmine has been empirically used for underactive bladder dysfunction in the hope of reducing residual urine, but the use of these drugs has not been standardized, due to lack of efficacy and serious side effects. The main reasons for these side effects may likely be due to their nicotinic effects.

Pilocarpine promotes physiological salivation by binding the muscarinic M₂ receptor in the salivary glands, and has been used to treat dry mouth [5]. The present study investigated whether this drug is effective for activation of urinary bladder via M₃ receptors. Because it is difficult to obtain human bladder tissues, we have only tested the effects of darifenacin as a muscarinic antagonist. We used pig urinary bladder because it has similar characteristics physiologically and pharmacologically to human urinary bladder for characterization of muscarinic receptor subtypes [6,7].

In the present study, pilocarpine produced contraction of the pig and human bladder with high potency. In the study with muscarinic antagonists, the affinity of M₃-receptor subtype antagonist was the highest on CRCs to pilocarpine in the pig and human detrusor muscle. These results were similar to those of CRCs to carbachol from a previous study, indicating that pig and human detrusors were mediated by M₃-receptors and that pilocarpine has similar potency to carbachol [6,7].

A limitation in our study was an insufficiency of human urinary bladder tissue samples, which were difficult to obtain for ethical reasons. This limited us to study only darifenacin to test muscarinic antagonist activity against CRCs to pilocarpine in human bladder tissue. We selected darifenacin because it has the highest selectivity for M₃ receptor among anti-muscarinic drugs that are used clinically for the treatment of overactive bladder.

The effects of pilocarpine for detrusor contraction should be investigated in clinical study. Therefore, we are now studying the effects of pilocarpine for the treatment of patients with detrusor underactivity.

**Conclusion**

Pilocarpine appears to produce contraction of the pig and human bladder through activation of M₃-muscarinic receptor.

**References**