



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

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### 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% O<sub>2</sub> and 5% CO<sub>2</sub>. 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) (M<sup>3</sup> selective antagonist), pirenzepine (M<sup>1</sup> selective antagonist), methoctramine (M<sup>2</sup> 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 pEC<sub>50</sub> 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 pA<sup>2</sup> 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 pA<sup>2</sup> 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 M<sup>3</sup>-muscarinic receptor.

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

### Introduction

Contraction of urinary bladder is mediated by activation of muscarinic receptors, with M<sub>3</sub>-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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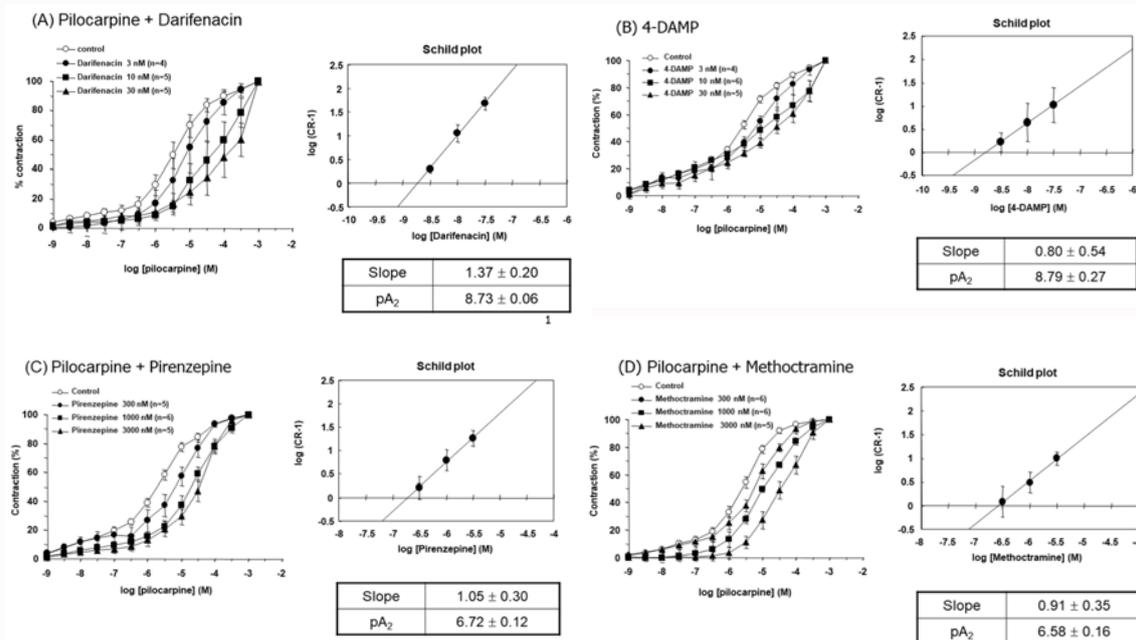
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**Figure 1:** Effects of darifenacin (A), 4-diphenyl acetoxy-methyl piperidine methiodide (4-DAMP) (B), pirenzepine (C), and methoctramine (D) on concentration-response curves to pilocarpine and Schild plot in pig bladder.

Muscle strips were mounted in 10-ml organ baths containing Krebs solution (composition in mM: NaCl 118.4, KCl 4.7,  $CaCl_2$  1.9,  $NaHCO_3$  24.9,  $MgSO_4$  1.15,  $KH_2PO_4$  1.15, glucose 11.7), which was maintained at 37°C and continuously gassed with 95%  $O_2$  and 5%  $CO_2$ . 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) ( $M_3$  selective antagonist), pirenzepine ( $M_1$  selective antagonist), or methoctramine ( $M_2$  selective antagonist) was used as muscarinic antagonist, and these muscarinic receptor antagonists were treated for 30 min before the addition of pilocarpine.

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.

## Statistical Analysis

The agonist potency and maximum response were expressed as

the mean  $pEC_{50} \pm$  Standard Error of the Mean (SEM) (-logarithm of the molar concentration of agonist resulting in 50% of the maximum response) and the mean maximum contraction  $\pm$  SEM, respectively. The  $pA_2$  value for a muscarinic receptor antagonist (determined as the x-intercept on the Schild regression plot) was only measured when the Schild plot slope was unity. Data were normalized for the maximal response on the control curve, and are expressed as the mean  $\pm$  SEM.

## Drugs and Chemicals

4-DAMP, methoctramine, and pirenzepine were purchased from Sigma Chemical (St. Louis, MO, USA). Pilocarpine and darifenacin were kind gifts from Kissei Pharmaceutical co., Ltd., (Matsumoto, Japan) and Pfizer Inc. (New York, USA), respectively.

## Results

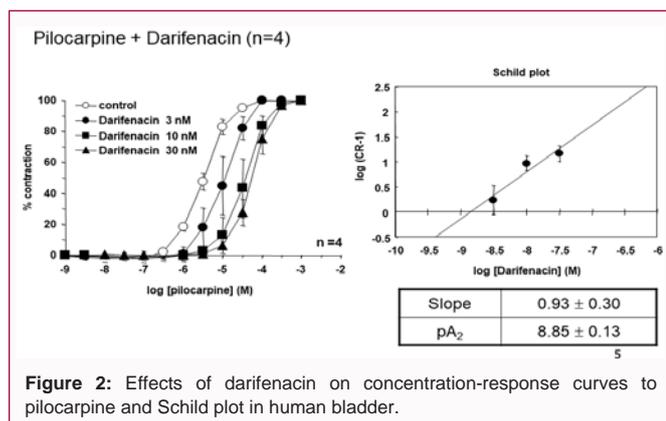
Pilocarpine induced contractions of smooth muscle of the detrusor in a concentration-dependent manner, with maximum contraction relative to 80 mM KCl of  $134.4 \pm 22.3\%$  and  $77.7 \pm 36.2\%$ , respectively, and  $pEC_{50}$  values of  $5.28 \pm 0.2$  and  $5.10 \pm 0.16$ , respectively, in the pig and human bladder.

Darifenacin, 4-DAMP, pirenzepine, and methoctramine caused surmountable antagonism of responses to pilocarpine, with Schild plot slopes of  $1.37 \pm 0.20$ ,  $0.80 \pm 0.54$ ,  $1.05 \pm 0.30$ , and  $0.91 \pm 0.35$ , respectively, in the pig bladder. The rank order of mean  $pA_2$  values was as follows: 4-DAMP ( $8.79 \pm 0.27$ ) = darifenacin ( $8.73 \pm 0.06$ ) > pirenzepine ( $6.72 \pm 0.12$ ) > methoctramine ( $6.58 \pm 0.16$ ) (Figure 1A-1D).

Darifenacin caused surmountable antagonism of responses to pilocarpine, with Schild plot slope of  $0.93 \pm 0.30$  and  $pA_2$  value of  $8.85 \pm 0.13$  in the human bladder (Figure 2).

## Discussion

For the management of voiding difficulty in patients with an



**Figure 2:** Effects of darifenacin on concentration-response curves to pilocarpine and Schild plot in human bladder.

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 M<sub>3</sub> 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* M<sub>3</sub> 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 M<sub>3</sub>-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 M<sub>3</sub>-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 M<sub>3</sub> 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 M<sub>3</sub>-muscarinic receptor.

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