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Antimuscarinic actions on bladder urothelium and lamina propria contractions are similar to those observed in detrusor smooth muscle preparations

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Abstract

Objectives: Antimuscarinic medications are the first-line treatments for overactive bladder, the most common form of bladder dysfunction. Their primary action is thought to block detrusor muscarinic receptors. It is unclear, however, if these therapeutics have actions on other tissues within the lower urinary tract. This study assessed whether clinical antimuscarinics have a functional impact on urothelium with lamina propria (U&LP) tissue.

Methods: Strips of porcine detrusor and U&LP were mounted in carbogen-gassed Krebs-bicarbonate solution at 37°C. The tissues were paired with carbachol-response curves performed in the absence or presence of each antimuscarinic. pEC50 values for each curve were analyzed and estimated affinities calculated.

Results: Both detrusor and U&LP tissues contracted with muscarinic receptor stimulation, which was inhibited by commonly used antimuscarinics. In detrusor samples ($p < 0.001$ for all), right parallel shifts from the control were observed in response to oxybutynin (1 μM), solifenacin (1 μM), tolterodine (1 μM), darifenacin (100 nM), trospium (100 nM) and fesoterodine (100 nM). This shift was consistent in U&LP samples, with no significant differences between the two layers.

Conclusions: The data suggests that clinical antimuscarinics are as effective at inhibiting tonic contractions of the U&LP as they are on detrusor, presenting the U&LP as an alternate target of these medications in the treatment of lower urinary tract symptoms.

KEYWORDS
detrusor, muscarinic antagonists, overactive bladder, therapeutic targets, urinary bladder, urothelium

1 | INTRODUCTION

Despite antimuscarinics being the gold standard pharmaceutical treatment for overactive bladder (OAB), more than 70% of patients who are administered this drug class cease their treatment regimens within a year.[1,2] The reason for this is not well understood, however, it is thought to be due to adverse side effects or lower-than-expected therapeutic benefits. The most commonly prescribed antimuscarinics for OAB worldwide include oxybutynin, solifenacin, tolterodine, darifenacin, fesoterodine, and trospium.[1,3] There is commonly a preference towards prescribing oxybutynin, solifenacin, tolterodine, and darifenacin,[4] in particular, as these exhibit reportedly fewer side effects and better symptom relief than the other options.[5,6]

The main region of smooth muscle for contractile activity, the detrusor, has formerly been the primary focus for the development of treatments for lower urinary tract disorders.[7] This has been supported by a clear mechanism of action for antimuscarinics to block cholinergic transmission within the detrusor tissue layer.[1,2] However, there have also been suggestions of anticholinergic activity in the urothelium with lamina propria (U&LP)[8] layer and it appears that the clinical antimuscarinics are likely to bind to the muscarinic receptors on the surface of cells within the U&LP.[9] However, to what extent pharmaceutical treatments focussing on this layer would impact the overall contractile activity of the tissue is unknown.

Having an action on the U&LP might present an important additional target for clinical antimuscarinics. There is evidence that highlights the release of neurotransmitters from the U&LP to influence bladder contraction.[10–12] This release, with acetylcholine being of particular importance, may occur in response to stretch,[8] identifying that a potential dysfunction of this tissue layer might contribute to bladder contractile disorders such as OAB. The U&LP can also endogenously release acetylcholine[13] which would also influence overall bladder contractions. A greater understanding on the factors influencing contraction in the urinary bladder, particularly across the different layers, is needed. It appears that clinically prescribed antimuscarinics are likely to bind to cells within the U&LP,[9] where they may influence the tissue's activity.[14] However, to what extent this would impact the actual contractile activity is unknown. This study aims to measure the functional responses to common antimuscarinics in the U&LP and compare these to those found in the detrusor.

2 | METHODS

2.1 | Tissue and organ bath preparation

Tissues were sourced from juvenile Large White-Landrace pigs (aged approximately 6 months). Porcine bladders are a well-established urinary bladder animal model with a number of previous studies demonstrating a structure and function similar to the human bladders.[10,11,15–18] Experiments were conducted on the day of animal slaughter, with two adjacent strips taken from the same bladder and paired together as control-experimental tissues. For both detrusor and U&LP preparation, these strips were sized as 1 cm height with 1 cm width. All dissections were done with frequent washing using ice-cold Krebs-Henseleit bicarbonate solution ("Krebs"): NaCl 118.4 mM, $C_6H_{12}O_6$, 11.7 mM, $NaHCO_3$ 24.9 mM, KCl 4.6 mM, $MgSO_4$ 2.41 mM, KH_2PO_4 1.18 mM and $CaCl_2$ 1.9 mM. Animal Ethics Approval was not required, as bladders were obtained from the local abattoir after slaughter for the routine commercial provision of food with no animals bred, harmed, culled, interfered, or interacted with as part of this research project.[19]

The U&LP were separated from the detrusor smooth muscle using surgical-grade fine scissors. The process employed follows past studies, using histology to confirm the viability of dissection for separation of these distinct layers[20]. Two separate tissues were dissected longitudinally from the wall of the bladder dome from each unique animal. As such, although individual strips are quoted in the methods (n values), the number of unique animals can be calculated as $n/2$ for each experiment.

Paired tissues were vertically hooked, and an isometric force transducer measured tension in an isolated tissue bath with 6 mL Krebs solution and carbogen gas (95% oxygen and 5% carbon dioxide) at 37°C. All tissues were equilibrated to a baseline of 2 g of tension. Both tonic contractions and spontaneous phasic contractions were recorded by the Powerlab system using Labchart version 8 (MCT050/D; ADInstruments). It took an average of 20 min for tissues to stabilize, with tissues then washed three times with warmed Krebs before the addition of any drugs.

2.2 | Pharmaceutical agents

Clinical antimuscarinics selected for this experiment were chosen as those most commonly prescribed for overactive bladder. Preliminary experiments assessed the inhibition of each antimuscarinic at three doses,

10 nM, 100 nM, 1 μM. In some cases, 1 μM resulted in a strong inhibition, where the tissue did not reach maximum contraction within the experimental time frame. When this occurred, the final concentration of the antimuscarinic used in the organ bath was reduced to 100 nM, to ensure complete concentration-response curves in response to carbachol. Of these, all reached maximum contraction at 100 nM, and no antimuscarinic was used at 10 nM. Final concentrations of the antimuscarinic in the functional organ bath were oxybutynin (1 μM), solifenacin (1 μM), tolterodine (1 μM), darifenacin (100 nM), fesoterodine (100 nM), and trospium (100 nM). Carbamoylcholine chloride (carbachol) was used as the agonist for a concentration-response curve. All antimuscarinics were sourced from Sapphire Bioscience (New South Wales, Australia) and the carbachol was sourced from Sigma-Aldrich (Missouri, U.S.). Oxybutynin, trospium and fesoterodine was dissolved in distilled water Solifenacin, tolterodine and darifenacin was dissolved in dimethyl sulfoxide and diluted using distilled water. For control tissues, an identical volume of vehicle was pipetted into the bath without any dissolved antimuscarinic. Carbachol was dissolved and diluted in Krebs solution.

2.3 | Measurements

Baseline tension was measured as the average reading of the tissue's resting tension before the addition of carbachol. After each cumulative dose of carbachol, the tension was recorded at the peak level of contraction. At the conclusion of the experiment, tissues were removed from each organ bath and weighed.

2.4 | Data analysis

All data was graphed and analyzed using GraphPad Prism version 9.5 MacOS (GraphPad Software). The specific analyzes used includes using a paired Student's two-tailed *t*-test for comparing the mean pEC50s between control and intervention groups to determine the different tissue functional response. From tests that showed significance, the mean estimated pKD and standard deviation(SD) were obtained. All data had a normal distribution, and as such, parametric tests were applied. A comparison between the detrusor and U&LP response was performed, using an unpaired Student's two tailed *t*-test. For all Student two tailed *t*-tests performed, significance was deemed $p < 0.05$.

3 | RESULTS

3.1 | Response to muscarinic receptor stimulation

Carbachol was added as grams per milliliter (and molar concentrations calculated after), with each cumulative dose increased the tissue's tension.

For detrusor preparations, carbachol concentrations of 910 nM increased tension ($n = 53$ for all) from the resting baseline of (mean ± SD) 1.68 ± 0.75 g to 1.97 ± 0.84 g, 2.73 μM increased tension to 2.58 ± 1.16 g, 9.11 μM increased tension to 5.3 ± 2.77 g, 27.23 μM increased tension to 6.21 g ± 3.18 g. Further concentrations of carbachol were added (91.1 μM, 273 μM, 910 μM, 2.73 mM, and 9.10 mM) but the tissue had reached maximum contraction, and no further tension increases were observed.

For U&LP preparations, carbachol concentrations of 910 nM increased tension ($n = 69$ for all) from the resting baseline (mean ± SD) of 1.87 ± 0.39 g to 2.43 ± 0.63 g, 2.73 μM increased tension to 3.23 ± 1.13 g, 9.11 μM increased tension to 4.11 ± 1.41 g, 27.23 μM increased tension to 4.97 ± 1.52 g, 91.1 μM increased tension to 5.39 ± 1.51 g. Further concentrations of carbachol were added (273 μM, 910 μM, 2.73 mM and 9.10 mM) but the tissue had reached maximum contraction, and no further tension increases were observed.

The average weight (mean ± SD) of the detrusor smooth muscle strips was 90 ± 50 mg ($n = 53$ for all), and the U&LP was 80 ± 40 mg ($n = 69$). For concentrations above 2.73 μM of carbachol, the detrusor contracted with more force than the U&LP tissues, reaching a higher baseline tension from each administration of carbachol.

3.2 | Inhibition of contraction from clinical antimuscarinics

Compared to responses recorded to carbachol in their paired control tissues, all antimuscarinics produced parallel shifts to the right in detrusor ($p < 0.01$, paired two-tailed Student's *t*-test). The estimated mean pKD values (mean with SD) were calculated for oxybutynin (7.47 ± 0.89, $n = 10$), solifenacin (7.87 ± 0.30, $n = 8$), tolterodine (8.09 ± 0.54, $n = 8$, Figure 1), darifenacin (7.58 ± 0.77, $n = 11$), fesoterodine (8.67 ± 0.68, $n = 8$) and trospium (8.69 ± 0.99, $n = 8$, Figure 2).

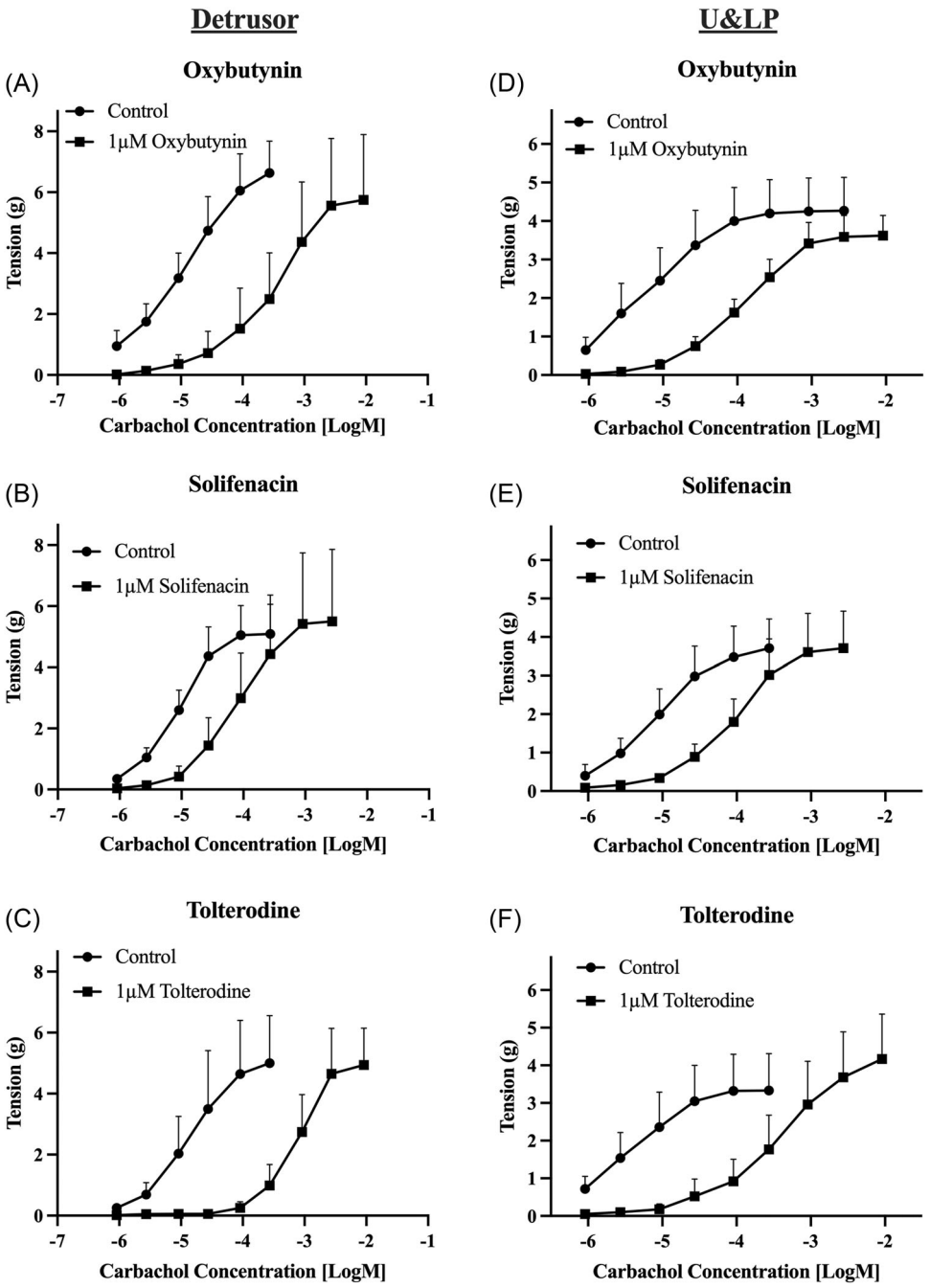

FIGURE 1 Detrusor carbachol concentration response curves in the presence and absence of (A) oxybutynin (1 μM, $n = 10$), (B) solifenacin (1 μM, $n = 8$), (C) tolterodine (1 μM, $n = 8$). U& LP carbachol concentration response curves in the presence and absence of (D) oxybutynin (1 μM, $n = 18$), (E) solifenacin (1 μM, $n = 10$), (F) tolterodine (1 μM, $n = 10$). Datapoints are represented as mean with 95% confidence interval.

All antimuscarinics, produced parallel shifts from the control in the U&LP ($p < 0.001$, paired two-tailed Student's *t*-test for each). The estimated U&LP pKD values (mean with SD) were calculated for oxybutynin (7.35 ± 0.71, $n = 18$), solifenacin (7.09 ± 0.66, $n = 10$), tolterodine (8.17 ± 0.56, $n = 10$, Figure 1), darifenacin (7.73 ± 0.63, $n = 10$), fesoterodine (8.41 ± 0.18, $n = 11$) and trospium (8.83 ± 0.35, $n = 10$, Figure 2).

3.3 | Comparisons of antimuscarinics efficacy between U&LP and detrusor

The response of detrusor and U&LP to each antimuscarinic was compared using the calculated estimated pKDs. The estimated pKD values were not significantly different between inhibitions observed for the detrusor and U&LP to each antimuscarinic (unpaired Students two-tailed *t*-test, Figure 3).

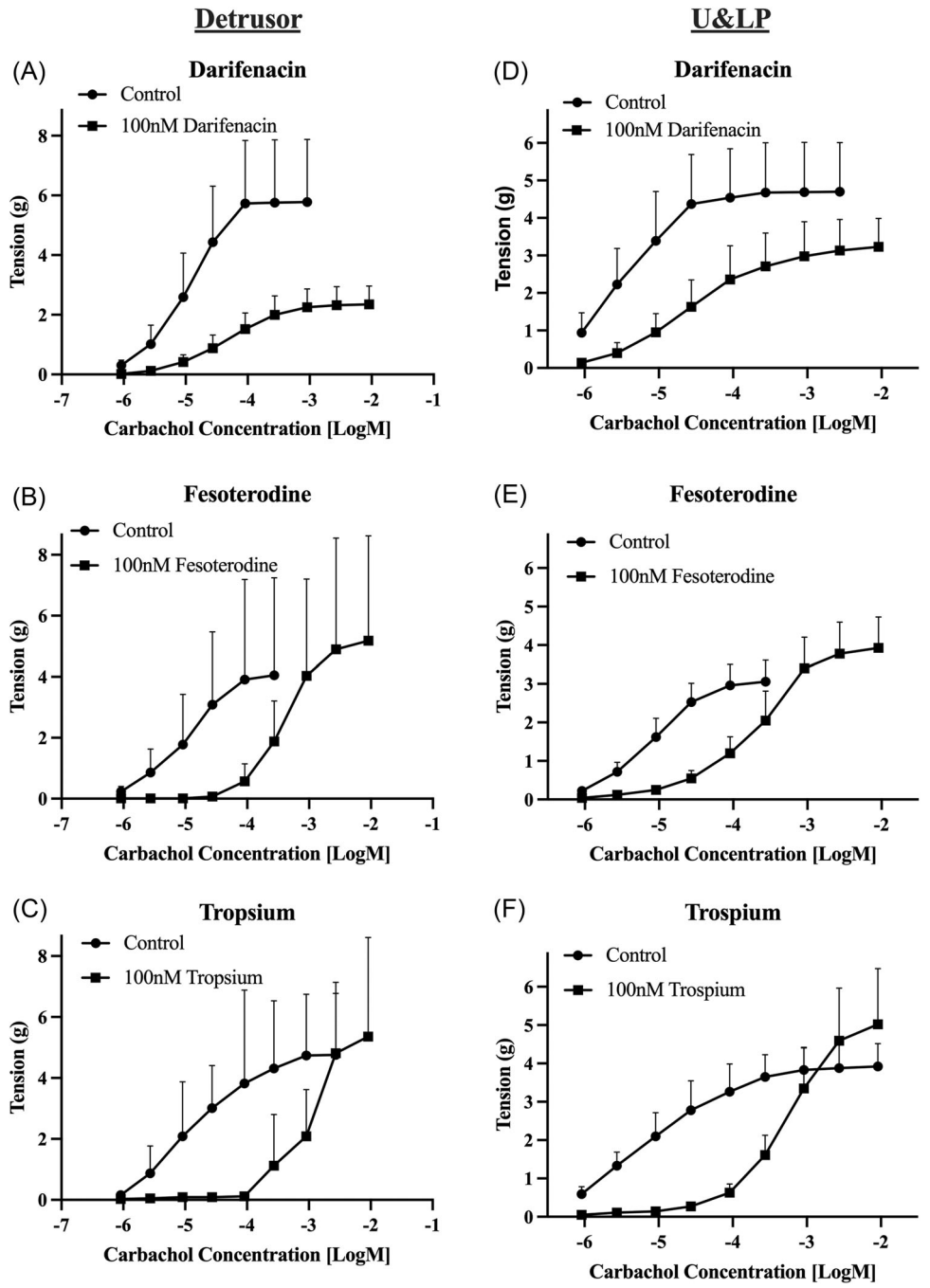

FIGURE 2 Detrusor carbachol concentration response curves in the presence and absence of (A) darifenacin (100 nM, $n = 11$), (B) fesoterodine (100 nM, $n = 8$), (C) trospium (100 nM, $n = 8$). U&LP carbachol concentration response curves in the presence and absence of (D) darifenacin (100 nM, $n = 10$), (E) fesoterodine (100 nM, $n = 11$), (F) trospium (100 nM, $n = 10$). Datapoints are represented as mean with 95% confidence interval.

4 | DISCUSSION

The increasing interest into the activity of the U&LP for the modulation of overall bladder activity,[8] and the finding in our study that these medications also have an impact on the U&LP in a similar way to the detrusor, presents a unique perspective for the development of future treatments.

Of the five subtypes of muscarinic receptors, the receptor most responsible for contraction are M3 receptors, which influences contraction within the detrusor. This subtype of muscarinic receptors can also contribute to bladder overactivity, thus, antimuscarinic selectivity towards this receptor are thought to have more favorable patient outcomes.[21] There is increased patient

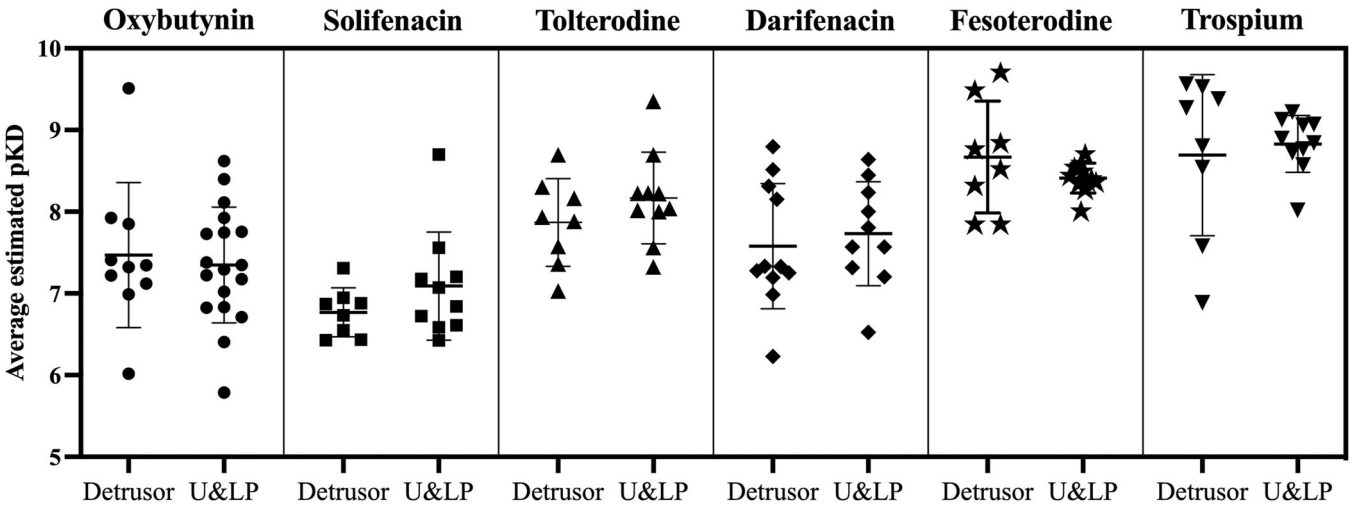

FIGURE 3 Comparison of estimated average pKD of oxybutynin (1 μM; detrusor $n = 10$; U&LP $n = 18$), solifenacin (1 μM; detrusor $n = 8$; U&LP $n = 10$), tolterodine (1 μM; detrusor $n = 8$; U&LP $n = 10$), darifenacin (100 nM; detrusor $n = 11$; U&LP $n = 10$), fesoterodine (100 nM; detrusor $n = 8$; U&LP $n = 11$) and trospium (100 nM; detrusor $n = 8$; U&LP $n = 11$) between the detrusor and U&LP. Datapoints are represented as mean with SD.

compliance with new generation clinical antimuscarinics, and this is thought to be related to an increased selectivity on the detrusor M3 receptors, minimizing adverse effects from actions on other tissues. As the M3 receptors mRNA expression differs in the U&LP and detrusor, the functional response between layers to these antimuscarinics, was potentially quite distinct.[22] However, the results suggest a consistent response. The nonsignificant differences between the affinities for any of the common antimuscarinics demonstrates that though M3 expression may differ, the activity of M3 receptors in each layer is similar. Although only estimated affinity values were acquired in this study, based on cumulative concentration response curves to carbachol (acting on the M3 receptor), the results appear consistent with the published literature, with pKD values ranging between 7 and 9 for all. There is a paucity of data using functional or in vitro studies, but when comparing to results obtained from radioligand binding, the values obtained in this study had overlapping standard deviations with that observed in human bladder detrusor and U&LP preparations.[9]

The results highlight a clinically relevant and functionally important action of antimuscarinics on the U&LP layer when used in the treatment of lower urinary tract disorders. Though the U&LP may be influenced by other pathways, it can directly contract with a relatively strong force in response to muscarinic stimulation. The U&LP can also release endogenous acetylcholine in response to stretch, which acts back on the U&LP[8] and influence downstream effects on the detrusor layer.[23] As such, the findings of this study demonstrate that common clinical antimuscarinics, through inhibiting the activity of the U&LP tissue, could offer noticeable benefits to patients with disorders such as OAB, where spontaneous contractions occur during the filling phase. This aligns with clinical findings, as intravesical administrations of oxybutynin, where the antimuscarinic would come in direct contact to the U&LP, have shown benefits to patients suffering from detrusor overactivity and overactive bladder symptoms. These benefits include increasing bladder compliance,[24] maximum bladder capacity[25] and decreasing urinary frequency.[26]

A limitation of this study is the use of only a few concentrations of antimuscarinic to obtain a compilation of dose responses to obtain an estimation of each antimuscarinic's affinity rather than an estimated pKD. The clinical significance these results pose and further research within this field includes incorporating the mechanism of U&LP for better patient outcomes through improving future patient antimuscarinic compliance.

5 | CONCLUSION

As general OAB patient compliance to antimuscarinics is low and the health burden of OAB continues to increase, this study provides an insight into this drug classes' mechanism of action on the bladder as a whole. Rather than solely targeting the detrusor, antimuscarinic medications likely also impact the U&LP, where they would have a clear and significant effect on inhibiting contractions. This alternative tissue may be an overlooked, yet important target of these drugs in the pharmacological

management of lower urinary tract symptoms. The development of future treatments could consider this identified action of clinical antimuscarinics on the U&LP as a relevant and important potential target that could alleviate and impact some forms of abnormal contractile activity in urinary bladder.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available by emailing corresponding author upon reasonable request.

ETHICS STATEMENT

Animal Ethics Approval was not required. All bladders were obtained from the local abattoir after slaughter for the routine commercial provision of food with no animals bred, harmed, culled, interfered, or interacted with as part of this research project.

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