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The Inhibitory Role of Acetylcholine and Muscarinic Receptors in Bladder Afferent Activity

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Abstract

Background: The main treatment for overactive bladder (OAB) is the use of anticholinergic drugs initially believed to inhibit the effect of parasympathetic acetylcholine (ACh) on the detrusor; however, there is now evidence to suggest that anticholinergic drugs could interact with sensory pathways.
Objective: Investigate the role of muscarinic receptors and ACh in modulating bladder afferent sensitivity in the mouse.
Design, setting, and participants: Bladder and surrounding tissue were removed from wild-type male mice, placed in a recording chamber, and continually perfused with fresh oxygenated Krebs solution at 35 °C. Bladders were cannulated to allow infusion and intravesical pressure monitoring, and afferent nerve fibres innervating the bladder were dissected and put into a suction electrode for recording.
Measurements: Multiunit afferent activity and intravesical pressure were recorded at baseline and during bladder distension. Experiments were conducted in the presence of muscarinic agonists and antagonist or in the presence of the cholinesterase inhibitor physostigmine.
Results and limitations: Blocking muscarinic receptors using atropine (1 μM) had no effect on spontaneous afferent discharge, the afferent response to bladder distension, or on bladder compliance. However, stimulation of muscarinic receptors directly using bethanechol (100 μM) and carbachol (100 μM) or indirectly using physostigmine (10 μM) significantly inhibited the afferent response to bladder distension and concurrently reduced bladder compliance. Furthermore, prior application of nifedipine prevented the changes in bladder tone but did not prevent the attenuation of afferent responses by bethanechol or physostigmine.
Conclusions: These data indicate that stimulation of muscarinic receptor pathways can depress sensory transduction by a mechanism independent of changes in bladder tone, suggesting that muscarinic receptor pathways and ACh could contribute to normal or pathologic bladder sensation.

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1. Introduction

Successful controlled emptying of the bladder depends on integrated autonomic and somatic neuronal mechanisms that coordinate relaxation of the urethral sphincters and concomitant contraction of the detrusor muscle. A disruption in these mechanisms can give rise to a number of lower urinary tract disorders, one of which is overactive bladder (OAB). It is widely recognised that bladder afferent nerves initiate the micturition reflex and ultimately regulate the cycle of bladder filling and bladder emptying.

Bladder afferent innervation is conveyed by the pelvic and hypogastric nerves arising from the lumbosacral and thoracolumbar dorsal root ganglia. These afferent nerves include myelinated Aδ-fibres, which are involved in normal micturition, and unmyelinated C-fibres, which are responsible for painful sensations [1]. Both mechanical and chemical stimuli can excite bladder afferents and trigger micturition [1,2].

Recent reports have indicated that anticholinergic drugs can relieve the storage symptoms associated with OAB such as urgency and frequency [3,4]. The presence of muscarinic receptors has also been detected throughout the urothelium [5–7], a structure believed to be an important component of sensory function [8], and there is evidence that stimulation of these receptors can alter the release of other transmitters and regulate bladder voiding reflexes [6,9,10]. This, along with the previously reported release of acetylcholine (ACh) from the urothelium [11], may indicate that muscarinic receptor pathways could be directly involved in afferent function of the bladder.

Understanding these pathways may hold the key to designing better pharmacologic agents to treat OAB. The aim of this study was to investigate the role of muscarinic receptors and endogenously released ACh in afferent excitability and mechanosensitivity.

2. Methods

2.1. Animals

Six- to eight-week-old C57/BL6 male mice were used in this study. Maintenance and killing of the animals followed principles of good laboratory practice in compliance with British national laws and regulations. All mice were humanely sacrificed by cervical dislocation.

2.2. Preparation and nerve recording

The whole pelvic region was dissected and placed in a recording chamber (volume: 30 ml) that was continually perfused with gassed (95% O_2, 5% CO_2) Krebs-bicarbonate solution (composition in mMol/l: NaCl 118.4, $NaHCO_3$ 24.9, $CaCl_2$ 1.9, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.7) at 35 °C. The urethra was catheterised and attached to an infusion pump, enabling the controlled distension of the bladder with saline. The dome was catheterised by a two-way cannula to enable recording of intravesical pressure and allow evacuation of fluid. The multiunit afferent nerve tracts consisting of pelvic and hypogastric nerves were dissected into fine branches, cut distal to the bladder, and placed in a suction electrode for recording. The electrical activity was recorded by a NeuroLog Headstage NL100 (Digitimer Ltd, Letchworth Garden City, UK), amplified, filtered (NL215; band pass: 300–4000 Hz), and captured by a computer via a power 1401 interface and Spike2 software v.5.14 (Cambridge Electronic Design, Cambridge, UK).

2.3. Experimental protocols

All preparations were allowed 30 min to stabilise before experiments commenced. Control bladder distensions were carried out using isotonic saline (0.9%) at a rate of 100 $\mu l/min^{-1}$ to a maximum pressure of 50 mm Hg, at which point the infusion pump was stopped and rapid evacuation of the fluid occurred by opening the two-way catheter in the dome to the atmosphere. This process was repeated several times at 10-min intervals to assess the viability and reproducibility of the preparation. Afferent responses to distension were then investigated in the presence of muscarinic receptor agonists and antagonists. All drugs were bath applied for 15 min. Bladder compliance was gauged from the pressure–volume relationship.

2.4. Data analysis and statistics

Whole-nerve multiunit afferent nerve activity was quantified by counting the number of action potentials crossing a preset threshold (Digitimer D130). The whole-nerve response to ramp distension was analysed as the change in afferent activity per unit of pressure, expressed as impulses per second (imp sec^{-1}) for the raw nerve activity or as a percentage of the maximum control response (immediately preceding distension). In all experiments, the afferent response to distension following application of a pharmacologic agent was expressed as a percentage of the maximal response of the preceding control distension, allowing time-matched paired comparisons to be made. Data are presented as means plus or minus the standard error of the mean. Statistical analysis was carried out using either a two-way analysis of variance (ANOVA) and Bonferroni post test or a Student t test where appropriate, and significance was assumed at $p < 0.05$.

2.5. Drugs

Atropine, 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP), pirenzepine, carbamylcholine chloride (carbachol), carbamyl-β-methylcholine chloride (bethanechol), and physostigmine (Sigma-Aldrich, Dorset, UK) were dissolved in distilled water, and nifedipine (Sigma-Aldrich, Dorset, UK) was dissolved in ethanol. All drugs were diluted in Krebs solution to required concentrations before use. As nifedipine is light sensitive, experiments were conducted in a darkened room. All salts were purchased from BDH (Poole, UK).

3. Results

3.1. The contribution of muscarinic receptors to bladder mechanosensitivity

Ramp distension of the bladder caused a progressive increase in intravesical pressure and afferent nerve discharge (Fig. 1A). Both afferent discharge and compliance remained reproducible with repeated control distensions (Fig. 1A and B). Blocking muscarinic receptors with the nonselective muscarinic antagonist atropine (1 μM; $n = 6$) had no significant effect on baseline spontaneous afferent discharge or responses to distension (Fig. 2).