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Effects of the M3 Receptor Selective Muscarinic Antagonist Darifenacin on Bladder Afferent Activity of the Rat Pelvic Nerve

Kazuyoshi Iijima, Stefan De Wachter, Jean-Jacques Wyndaele *

Department of Urology, Faculty of Medicine, University Antwerp, Antwerp, Belgium

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Abstract

Objective: Previous studies have revealed that intravesical and systemic administration of oxybutinin suppress pelvic afferent nerves. This study evaluates the efficacy of a selective M3 antimuscarinic, darifenacin, on bladder afferent activity.

Methods: Sixteen single bladder afferent fibers were isolated in nine female Sprague-Dawley rats. On the basis of their conduction velocities, they were grouped as Aδ or C fibers. The effect of repeat bladder filling was studied on the mechanosensitive properties of these units. The M3 receptor selective muscarinic antagonist darifenacin (0.1 mg/kg) was administered intravenously. Unitary afferent activity was again analyzed 30, 60, 90, and 120 min after the drug administration.

Results: Seven units corresponded to criteria for Aδ fibers, nine for C fibers. Repeat bladder filling did not change nerve activity in Aδ or C fibers. When nerve activity was expressed as a percent of control activity, afferent sensitivity changed after darifenacin in Aδ fibers: $86 \pm 27\%$, 30 min ($p > 0.05$), $69 \pm 32\%$, 60 min ($p < 0.05$), $56 \pm 36\%$, 90 min ($p < 0.05$), and $61 \pm 49\%$, 120 min ($p > 0.05$), and in C fibers: $70 \pm 39\%$, 30 min ($p < 0.05$), $57 \pm 49\%$, 60 min ($p < 0.05$), $45 \pm 42\%$, 90 min ($p < 0.01$), and $47 \pm 43\%$, 120 min ($p < 0.01$).

Conclusions: In this study we show that darifenacin reduces bladder afferent activity in both Aδ and C fibers. The decrease in afferent spikes in C fibers may be more pronounced than that in Aδ fibers. These results may explain that the efficacy of darifenacin in overactive bladder symptoms is partly due to bladder afferent desensitization.

* Corresponding author. Department of Urology, University Hospital Antwerp, 10, Wilrijkstraat, B-2650 Edegem, Belgium. Tel. +32 (0) 38213511; Fax: +32 (0) 38214479.
E-mail address: Jean-Jacques.Wyndaele@uza.be (J.-J. Wyndaele).

1. Introduction

Overactive bladder (OAB) is a highly prevalent symptom complex defined as ''urgency, with or without urgency incontinence, usually with frequency and nocturia'' [1,2].

Recent findings have suggested that, during the storage phase, there is an ongoing release of acetylcholine acting on muscarinic receptors from nerves and/or urothelium [3,4]. Acetylcholine may act directly on afferent nerves to initiate the micturition reflex and contribute to the symptoms of OAB. If this is correct, inhibition of acetylcholine release or blockade of the postjunctional muscarinic receptors could be expected to reduce bladder afferent firing during storage and to increase bladder capacity. Thus, antimuscarinics are thought to be the drugs of choice for treatment of OAB [5,6]. However, the side-effects of antimuscarinics, especially dry mouth, constipation, and blurred vision, limit their use. There have been advances in the understanding of muscarinic receptors and bladder function, which have lead to the search for subtype selective antimuscarinic agents with improved tolerability [7,8].

Darifenacin is a widely used drug for OAB; its efficacy, tolerability, and safety have been proven [9–11]. Darifenacin has been shown to have high affinity and selectivity for the muscarinic M3 receptor, with low selectivity for the other muscarinic receptor subtypes, which is expected to have clinical efficacy in OAB with fewer adverse events related to the blockade of other muscarinic receptor subtypes.

Recently, it has been shown that afferent C fibers have temporally decreased activity after intravesical administration of oxybutynin (a nonselective antimuscarinic) [12,13], and that both Aδ and C fibers have significantly decreased activity after systemic administration [14]. However a direct effect of darifenacin on bladder afferent activity has not been shown. This study aims to evaluate the efficacy of a selective M3 antimuscarinic on afferent bladder activity by recording its effects on single-fiber afferent activity of the pelvic nerve of the rat.

2. Methods

Nine female Sprague-Dawley rats (250–350 g) were used. The animals were anesthetized with urethane (1.5 g/kg ip). To maintain deep anesthesia, supplemental doses were administrated if necessary. The trachea and jugular vein were cannulated after a midline neck incision. Body temperature was maintained by a heated blanket. After the experiments, the animals were sacrificed by an overdose of urethane.

The protocol was approved and carried out in accordance with the institutional ethical committee guidelines for animal research.

The pelvic structures were exposed by a left flank incision. The ureters were ligated close to the bladder. The left pelvic nerve was cleared from surrounding tissue proximal to the major pelvic ganglion. A pair of Teflon-coated silver electrodes were placed around the pelvic nerve and sealed with Wacker Silgel (Wacker Chemie, Munich, Germany). A three-barrel catheter was inserted into the bladder dome and secured by suture. One barrel was used to fill and empty the bladder, and was attached to a pressure transducer. In each of the other two barrels, a Teflon-coated silver wire electrode was inserted for bipolar intravesical electrical stimulation. The urethra was ligated to allow accurate comparison of afferent nerve activity with intravesical pressure. To reduce background somatic afferent activity, we cut the sciatic nerve and all nerves at the base of the tail.

Then the animal was placed prone, the lumbosacral spinal cord was exposed by laminectomy, and the dura mater was opened. Both L6 dorsal roots were cut close to their entrance to the spinal cord. The dorsal skin was tied up to make a pool, which was filled with body warm paraffin oil.

Fine filaments were dissected from the left L6 dorsal root and placed across shielded bipolar silver electrodes. Recorded nerve activity was preamplified with a low-noise AC differential amplifier (10×) and filtered (60–5000 Hz). A final amplification (10,000×) was used before the activity was displayed on an oscilloscope. Afferent fibers originating from the bladder were identified both by electrical stimulation (0.5-ms square wave pulses) of the pelvic nerve and by intravesical electrical stimulation. The filaments were teased until a maximum of three clearly different unitary action potentials were evoked by electrical stimulation. These action potentials were discriminated by the Spike2 (CED, Cambridge, England) impulse shape recognition program. Nerve activity was sampled at 20 kHz and bladder pressure at 100 Hz with the data acquisition program. Conduction velocities (CVs) were calculated from the latency of response to electrical stimulation and the conduction distance.

Fibers were grouped on the basis of conduction velocities. Those with a CV < 2.5 m/s were considered to correspond with unmyelinated C fibers and those with a CV > 2.5 m/s with thinly myelinated Aδ fibers [12,14,15].

Single-fiber afferent activity was recorded during constant flow cystometry with body-warm saline at a filling rate of 80 μl/min, which is within the range of physiologic filling rates [16]. Filling continued until an intravesical pressure of 35 cm water was reached. At the beginning of the experiments, three cystometry studies were performed at 30-min intervals to evaluate the reproducibility of voiding threshold volumes and pressures, and of unitary afferent activity. The third cystometry served as the control observation for the following fillings.

Pure powder darifenacin solved in saline at a dose of 0.1 mg/kg was administered intravenously. Cystometrical parameters and afferent activity were again assessed 30, 60, 90, and 120 min after drug administration. Because we have already shown in the previous time-matched parenteral saline