Neurourology and Urodynamics 25:156–161 (2006)

Systemic Oxybutynin Decreases Afferent Activity of the Pelvic Nerve of the Rat: New Insights Into the Working Mechanism of Antimuscarinics

Kevin De Laet, Stefan De Wachter, and Jean-Jacques Wyndaele*
Department of Urology, Faculty of Medicine, University Antwerp, Edegem, Belgium

Aims: In a rat model, intravesical oxybutynin was recently shown to suppress pelvic afferent nerves. This study evaluates if a similar effect exists after systemic administration of oxybutynin. **Methods:** Twenty-four single afferent bladder nerves were identified in 15 rats. Based on their conduction velocities they were grouped as C or Aδ fibers. Bladder filling parameters and afferent nerve spike rate were simultaneously recorded 30 min before administration of saline (nine fibers) or oxybutynin (15 fibers, 1 mg/kg), and again 30, 60, 90, 120, and 150 min after systemic saline or drug administration. **Results:** No change in C or Aδ afferent spike rate was observed after saline injection ($P > 0.90$). In the study group, a decrease in afferent activity was noted after systemic administration of oxybutynin for C fibers, which were statistically significant 90 ($P < 0.004$) and 120 min ($P < 0.028$) after drug delivery. After 150 min, the spike rate was still lower compared to the baseline filling, without reaching the level of significance ($P > 0.09$). For the Aδ fibers the decrease in afferent spike rate was already significant at 30 min ($P < 0.005$) and remained significant during all subsequent fillings ($P < 0.012$). To avoid a possible confounding influence of the bladder compliance, which increased significantly after injection of oxybutynin ($P < 0.011$), afferent activity during bladder filling was recalculated. Normalized afferent sensitivity of C and Aδ fibers decreased significantly after injection of oxybutynin. This means that the decrease in afferent spike rate is not the result of an increased compliance. **Conclusions:** The findings of this study strongly suggest that oxybutynin directly or indirectly influences bladder sensory nerves, inhibiting the afferent part of the micturition reflex. *Neurourol. Urodynam. 25:156–161, 2006.* © 2005 Wiley-Liss, Inc.

Key words: anticholinergic; antimuscarinic; overactive bladder; oxybutynin; sensory

INTRODUCTION

Antimuscarinics are considered the first-line treatment for patients with overactive bladder syndrome [Andersson, 2004]. Although they have been extensively studied, the mechanism of action is incompletely understood. The clinical benefits are thought to be due to blocking the muscarinic receptors on the efferents in the detrusor smooth muscle, which are stimulated by activated parasympathetic fibers, thereby reducing the ability of the detrusor to contract [Chapple et al., 2002; Andersson, 2004]. However the clinical effects of antimuscarinics—i.e., a decrease in desire to void and an increase in bladder capacity [Yarker et al., 1995]—are observed during the storage phase, when there is normally no ongoing excitatory parasympathetic activity [Yoshimura and De Groat, 1997].

Oxybutynin is an antimuscarinic that is frequently used in clinical practice and is one of the most studied antimuscarinics. Recently, it has been shown that intravesical administered oxybutynin gives a temporary decrease in afferent activity in C-fibers [De Wachter and Wyndaele, 2003; Kim et al., 2005]. Although this effect has been attributed to the local anesthetic properties of oxybutynin, which is a drug with mixed actions, such anesthetic properties have been doubted after systemic administration [Andersson, 2004]. This study was set up to investigate the influence of systemically administered oxybutynin on afferent bladder activity, by recording its effects on single fiber afferent activity of the pelvic nerve of the rat.

MATERIALS AND METHODS

Fifteen female Sprague–Dawley rats (250–350 g) were used. The animals were anesthetized with urethane (1.5 g/kg IP) because urethane is well known to spare the micturition reflex [Matsuura and Downie, 2000]. To maintain deep anesthesia, supplemental doses were administrated if necessary. The trachea was cannulated allowing spontaneous

Grant sponsor: University of Antwerp.
*Correspondence to: Jean-Jacques Wyndaele, Department of Urology, University Hospital Antwerp, 10, Wilrijkstraat, B-2650 Edegem, Belgium.
E-mail: Jean-Jacques.Wyndaele@uza.be
Received 10 July 2005; Accepted 13 September 2005
Published online 21 December 2005 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/nau.20208

respiration. 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 was 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 be able to accurately compare afferent nerve activity with intravesical pressure. To reduce background somatic afferent activity the sciatic nerve and all nerves at the base of the tail were cut.

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 platinum electrodes. Recorded nerve activity was pre-amplified with a low noise AC differential amplifier (10×) and filtered (60−5,000 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 msec 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, bladder pressure at 100 Hz with the data acquisition program. The latency was measured for each afferent unit and the conduction velocity (CV) was calculated. Fibers were grouped based on conduction velocities. Those with a CV <2.5 m/sec were considered to correspond with unmyelinated C fibers and those with a CV >2.5 m/sec with thinly myelinated Aδ fibers [Sengupta and Gebhart, 1994].

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 physiological filling, rates [Pollock et al., 1986]. Filling continued until an intravesical pressure of 30 cm water was reached.

To determine the influence of oxybutynin, a placebo-controlled study design was used. In the study group, 0.5 ml of pure powder oxybutynin soluted in saline at a dose of 1 mg/kg was administered subcutaneously at the left shoulder, whereas in the placebo group, 0.5 ml of saline was injected. Cystometrical parameters and afferent activity were recorded 30 min before administration of oxybutynin or saline (baseline filling), and again assessed 30, 60, 90, 120, and 150 min after drug or placebo administration.

Unitary afferent activity was evaluated in relationship to intravesical pressure and volume. The relationship of nerve activity to pressure was established by comparing nerve activity and intravesical pressure at 1-sec intervals. These values were then averaged at 5 cm water intervals and average unitary activity was graphed as a function of intravesical pressure. To evaluate the relationship of nerve activity to intravesical volume, these values were compared at 1-sec intervals. The filling phase was then equally divided into five parts and the nerve activity per 1-sec was averaged for each part. The average unitary activity was graphed as a function of intravesical volume. Afferent nerve activity is expressed as impulses per second (i.e., spike rate).

For statistical analysis the MANOVA test for multiple comparisons was used. Averaged data are presented as the mean ± standard deviation (SD). Plots are presented as mean ± 95% confidence intervals. P values <0.05 are considered statistically significant.

RESULTS

The study group included 15 single unit afferent fibers isolated in 12 rats. Eight units were considered unmyelinated C fibers (CV: 1.61 ± 0.32 m/sec), seven myelinated Aδ fibers (CV: 5.5 ± 1.43 m/sec) [Sengupta and Gebhart, 1994]. In the control group, nine afferents were isolated in three rats: four C fibers (CV: 1.4 ± 0.28 m/sec) and five Aδ fibers (CV: 6.2 ± 1.23 m/sec).

In the control group, no change in afferent spike rate towards bladder pressure or volume was found between the baseline filling and subsequent fillings after systemic administration of saline, both in Aδ and C fibers ($P > 0.90$). The data on afferent activity towards bladder volume are illustrated in Figure 1. No difference was seen between baseline values of the control group and the study group ($P > 0.91$).

In the study group, a decrease in afferent activity was noted after systemic administration of oxybutynin both for Aδ and C fibers. Figure 2 shows raw tracings from an experiment on a C fiber.

Thirty minutes after oxybutynin delivery, the afferent spike rate in C fibers showed a tendency to decrease compared to the spike rate during baseline filling at corresponding bladder pressures and volumes. The decrease in spike rate was statistically significant 90 ($P < 0.004$) and 120 min ($P < 0.028$) after drug delivery. During the bladder filling after 150 min, the spike rate was still lower compared to baseline, without reaching the level of significance ($P > 0.09$). A decrease in afferent spike rate was also noted for the Aδ fibers with some statistical differences: at 30 min the decrease was already significant ($P < 0.005$) and remained significant during all

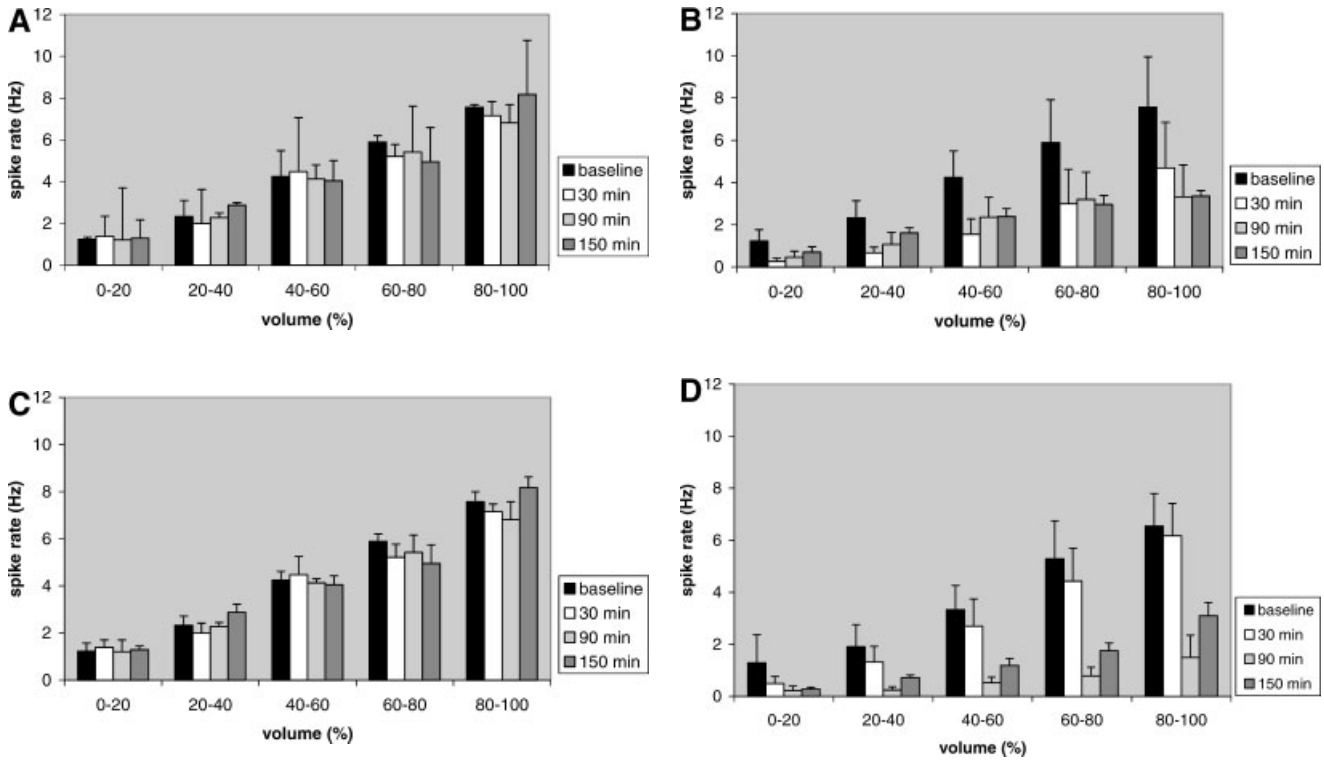

Fig. 1. **A**: Effect of saline on Aδ afferent activity. **B**: Effect of oxybutynin on Aδ afferent activity. **C**: Effect of saline on C afferent activity. **D**: Effect of oxybutynin on C afferent activity. Afferent spike rate at baseline, 30, 90, and 150 min is plotted against the bladder volume, which is divided into five equal parts. Data of 60 and 120 min are not shown. No significant difference is found between baseline fillings and fillings after saline injection in Aδ or C fibers (A and C). Significant decrease in afferent activity in Aδ fibers after 30, 90, and 150 min (B). Significant decrease in afferent activity in C fibers after 90 min, but not after 30 and 150 min (D).

subsequent fillings ($P < 0.012$). The data on afferent activity towards bladder volume are illustrated in Figure 1.

Bladder wall mechanoreceptors respond both to intravesical pressure and volume. Therefore afferent activity generated by the mechanoreceptors cannot be adequately studied solely in relation to pressure or volume, but should also be investigated towards the relationship between bladder pressure and volume [Vaughan and Satchell, 1995]. This relationship can be expressed as the bladder compliance. Because an increase in bladder compliance was previously shown after systemic administration of oxybutynin [Watanabe and Constantinou, 1996], compliance was calculated for all fillings. The data are listed in Table I. In the control group, no difference was seen between baseline and subsequent fillings ($P > 0.05$). In the study group, a significant increase in bladder compliance was noted between baseline and all fillings after administration of oxybutynin ($P < 0.011$).

To avoid a possible confounding influence of the change in compliance, the sensitivity of afferent fibers to respond to bladder filling was recalculated normalized for compliance. All cystometric filling curves were divided in five equal volume parts, and the change in afferent spike rate (i.e., the sensitivity of afferents to respond to filling) was then divided by the pressure change for that specific part of the filling curve. In the control group, no difference was found in afferent sensitivity normalized for compliance between the different bladder fillings. Figure 3 shows the effect of oxybutynin on filling sensitivity of Aδ and C fibers, normalized for bladder compliance. For the Aδ fibers, afferent sensitivity 30 min after oxybutynin was 47% ($P < 0.001$) of the baseline sensitivity, 40% after 60 min ($P < 0.001$), 25% after 90 min ($P < 0.001$), 45% after 120 min ($P < 0.01$), and 38% after 150 min ($P < 0.01$). For the C fibers, afferent sensitivity, respectively was 91% ($P > 0.05$), 67% ($P < 0.001$), 47% ($P < 0.001$), 42% ($P < 0.01$), and 51% ($P < 0.01$) compared with baseline. No difference was found between Aδ and C fibers after oxybutynin, except for the filling after 30 min, during which afferent sensitivity was significantly more reduced in Aδ than in C fibers ($P < 0.01$).

DISCUSSION

Oxybutynin is an antimuscarinic, spasmolytic, and local anesthetic drug [Andersson, 2004]. Previously, it has been shown that its effect on the guinea pig bladder in vivo was only significantly correlated with its antimuscarinic activity and not with its other actions [Noronha-Blob et al., 1989].

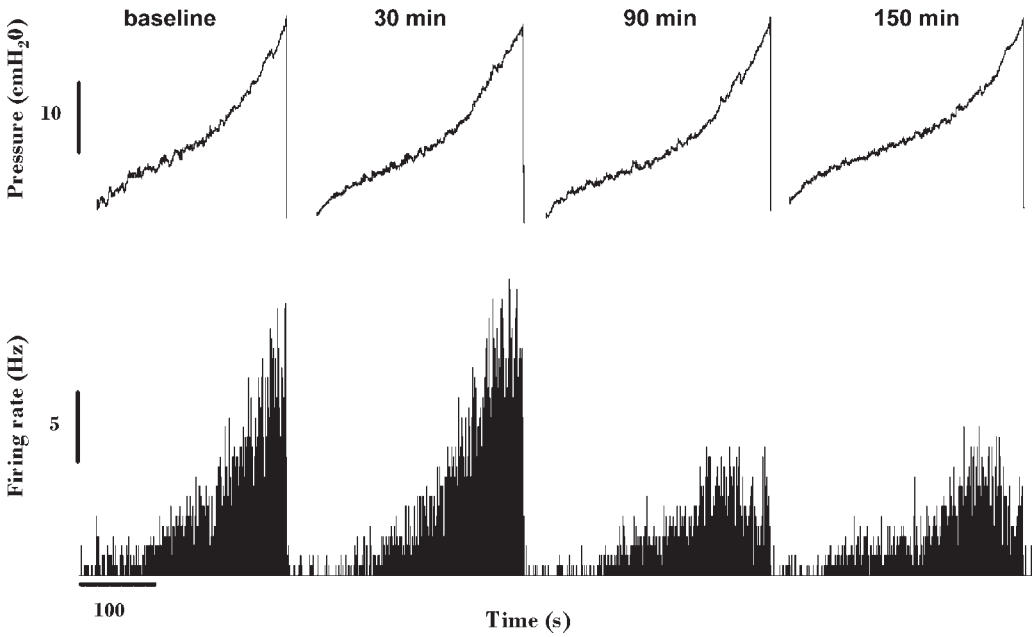

Fig. 2. Upper (from the **left** to the **right**): Cystometries before and 30, 90, and 150 min after systemic administration of oxybutynin. **Lower** (from the **left** to the **right**): Subsequent spike rate (Hz) of one afferent C fiber before and 30, 90, and 150 min after systemic oxybutynin. The afferent spike rate is clearly decreased after 90 min. Data of 60 and 120 min are not shown.

Therefore, the inhibition of parasympathetically induced detrusor contractions has generally been accepted as the main contributor to the clinical benefit of oxybutynin, by blocking muscarinic receptors in efferent fibers of the pelvic nerve in the detrusor smooth muscle [Chapple et al., 2002; Andersson, 2004]. Oxybutynin improves symptoms related to bladder filling [Yarker et al., 1995]. During the filling phase, there is increasing afferent activity in the pelvic nerve, but normally no ongoing excitatory parasympathetic activity [Yoshimura and De Groat, 1997]. Therefore, the effect of oxybutynin on the afferent fibers in the pelvic nerve in rats needed to be studied.

TABLE I. Data on Bladder Compliance (ml/cm Water) Calculated Between the Start and the End (Intravesical Pressure of 30 cm Water) of the Filling Phase

Time (min)	Control group	Study group
−30	0.014 ± 0.005	0.015 ± 0.006
0	—	—
30	0.014 ± 0.006	0.016 ± 0.007
60	0.014 ± 0.006	0.018 ± 0.007*
90	0.015 ± 0.007	0.020 ± 0.007*
120	0.014 ± 0.003	0.021 ± 0.006*
150	0.015 ± 0.005	0.025 ± 0.010*

Data presented are from the bladder filling before (−30 min) and the different recordings after subcutaneous injection of saline (control group) and oxybutynin (study group) (30, 60, 90, 120, and 150 min).
Data marked with an asterisk (*) are statistically significant.

This study shows that systemic administration of oxybutynin decreases the afferent spike rate during bladder filling. The decrease in spike rate was noted in both Aδ and C fibers, with some difference. In Aδ fibers the effect occurred earlier as the decrease in afferent spike rate was already significant 30 min after oxybutynin, whereas a significant decrease in spike rate was not seen up to 90 min after oxybutynin in C fibers. Afferent activity was not completely restored to baseline values after 150 min, which is not a surprise since the half life of oxybutynin is between 1 and 5 hr [Guay, 2003]. The decrease in afferent activity can be attributed to the effect of oxybutynin and not to repeated bladder fillings as no change was noted in

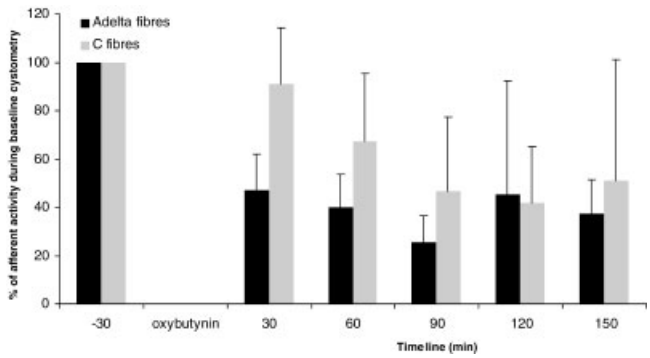

Fig. 3. Effect of oxybutynin on filling sensitivity of Aδ and C fibers after normalization for the increase in bladder compliance. Afferent activity decreases significantly after injection of oxybutynin, except for C fibers after 30 min.

afferent activity between baseline and consecutive fillings in the control group after injection of saline.

In the experimental group, a single dose was tested. In animal experiments, doses up to 0.3 mg/kg i.v. are used [Modiri et al., 2002]. For this study, a higher dose was chosen because a slower gradual release can be expected after subcutaneous administration, due to the distribution in fat.

Systemic administration of oxybutynin increased bladder compliance significantly, which is consistent with the study of Watanabe and Constantinou [1996] that analyzed the pressure/flow characteristics and their modulation in rats. The impact of compliance on afferent activity has to our knowledge not been studied, but it can easily be perceived that an increase in bladder compliance, leading to a reduced intravesical pressure for a given volume, might reduce afferent activity. To exclude a possible effect of a change in compliance on the afferent response after administration of oxybutynin, the change in spike rate over a given bladder volume interval was divided by the respective intravesical pressure increase, thereby enabling us to compare afferent sensitivity (i.e., change in afferent spike rate) at equal volumes and pressures between the bladder fillings at the different time recordings. In the control group, no difference was noted in afferent sensitivity normalized for bladder compliance, which was expected since no difference was found in compliance for the different bladder fillings. In the experimental group, afferent sensitivity after oxybutynin was reduced compared to baseline activity. These data show that the reduced afferent input to the central nervous system after oxybutynin is due to an effect on afferent fibers and not merely to changes in compliance. Why the effect on Aδ fibers comes earlier than on C fibers needs to be studied.

Recent studies in rats have also shown an inhibitory effect of oxybutynin when given intravesically on bladder afferents [De Wachter and Wyndaele, 2003; Kim et al., 2005], characterized by a temporary decrease in afferent spike rate in C fibers, without any effect on Aδ fibers [De Wachter and Wyndaele, 2003]. The observed effect of intravesical oxybutynin was attributed to its local anesthetic properties and the different influence on Aδ and C fibers to the penetration of the drug through the urothelium together with the different distribution of nerve endings from C fibers and Aδ fibers. After systemic administration, it is thought that the local anesthetic effect is less likely to be of importance [Andersson, 2004]. Furthermore, in our study the ureters were ligated before the start of the experiments to exclude a possible local anesthetic effect from oxybutynin or metabolites after renal excretion.

Therefore, the local anesthetic properties are probably not the mechanism involved in the inhibitory effect on the afferent fibers. Oxybutynin also has spasmolytic actions, but this is clinically probably not relevant as the in vitro smooth muscle relaxant activity is 500 times weaker than its antimuscarinic potency [Kachur et al., 1988]. Given these considerations, it seems most likely that the decrease in afferent activity is due to the antimuscarinic effect of oxybutynin.

The transduction from mechanical bladder stretch into afferent potentials regulating bladder sensory function is very complex and might involve different steps and mediators. During filling, several transmitters are released from the urothelium, which include acetylcholine, ATP, NO, and other transmitters [Ferguson et al., 1997; Vlaskovska et al., 2001; Andersson and Arner, 2004; Yoshida et al., 2004]. These transmitters may exert their effect directly on afferent endings, or indirectly by acting on the suburothelial myofibroblasts, which may modulate afferent activity through their communications with the urothelium, the afferent nerve endings and the detrusor smooth muscle [Andersson and Arner, 2004; De Groat, 2004; Yoshida et al., 2004]. Bladder stretch during filling induces urothelial release of ATP, which activates bladder afferents [Vlaskovska et al., 2001]. Acetylcholine, also released by the urothelium during filling [Yoshida et al., 2004] increases the release of ATP [Birder et al., 2003], which may facilitate the generation of afferent activity. Theoretically reducing the release of acetylcholine or preventing its action by blocking muscarinic receptors, found in the human and pig urothelium [Hawthorn et al., 2000], may reduce the afferent activity during bladder filling. This could explain the decrease in afferent activity and sensitivity found in this study after systemic administration of the antimuscarinic agent oxybutynin. This study does not permit to conclude whether oxybutynin acts directly on the afferent nerves or indirectly by blocking the release of acetylcholine in the urothelium, the interstitial cells or other related structures.

CONCLUSIONS

The common view on the working mechanism of systemic antimuscarinics such as oxybutynin was that they inhibit parasympathetically induced detrusor contractions through blocking the muscarinic receptors in the detrusor smooth muscle [Chapple et al., 2002; Andersson, 2004]. However, antimuscarinics are widely used in the overactive bladder because they improve symptoms, which are related to the storage phase, when there is normally no excitatory activity in parasympathetic efferent nerves [Yoshimura and De Groat, 1997]. The data in our study show that oxybutynin given systemically, reduces afferent spike rate and afferent sensitivity in both Aδ and C fibers. The reduced afferent input to the central nervous system may explain the observed sensory effects seen in clinical practice after administration of oxbytunin and possibly other antimuscarinic agents. The antimuscarinic drug oxybutynin acts on the peripheral part of the afferent limb of the micturition reflex, but the exact site of action within the bladder wall needs to be further determined. The results of this study do not permit making conclusions on the effect of other doses as only one dose was tested.

REFERENCES

Andersson KE. 2004. Antimuscarinics for treatment of overactive bladder. Lancet 3:46–3.

Andersson KE, Arner A. 2004. Urinary bladder contraction and relaxation: Physiology and pathophysiology. Physiol Rev 84:935–6.

Birder LA, Barrick SR, Roppolo JR, et al. 2003. Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol 285:F423–9.

Chapple CR, Yamanishi T, Chess-Williams R. 2002. Muscarinic receptor subtypes and management of the overactive bladder. Urology 60:82.

De Groat WC. 2004. The urothelium in overactive bladder: Passive bystander or active participant? Urology 64:7.

De Wachter S, Wyndaele JJ. 2003. Intravesical oxybutynin: A local anesthetic effect on bladder C afferents. J Urol 169:1892–5.

Ferguson DR, Kennedy I, Burton TJ. 1997. ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes—A possible sensory mechanism? J Physiol 505:503–11.

Guay DR. 2003. Clinical pharmacokinetics of drugs used to treat urge incontinence. Clin Pharmacokinet 42:1243–85.

Hawthorn MH, Chapple CR, Cock M, et al. 2000. Urothelium-derived inhibitory factor(s) influences on detrusor muscle contractility in vitro. Br J Pharmacol 129:416–9.

Kachur JF, Peterson JS, Carter JP, et al. 1988. R and S enantiomers of oxybutynin: Pharmacological effects in guinea pig bladder and intestine. J Pharmacol Ther 247:867–72.

Kim Y, Yoshimura N, Masuda H, et al. 2005. Antimuscarinic agents exhibit local inhibitory effects on muscarinic receptors in bladder-afferent pathways. Urology 65:238–42.

Matsuura S, Downie JW. 2000. Effect of anesthetics on reflex micturition in the chronic cannula-implanted rat. Neurourol Urodyn 19:87–99.

Modiri AR, Alberts P, Gillberg PG. 2002. Effect of muscarinic antagonists on micturition pressure measured by cystometry in normal, conscious rats. Urology 59:963–8.

Noronha-Blob L, Lowe VC, Peterson JS, et al. 1989. The anticholinergic activity of agents indicated for urinary incontinence is an important property for effective control of bladder dysfunction. J Pharmacol Exp Ther 251:586–93.

Pollock DM, Butterfield MI, Ader JL, et al. 1986. Dissociation of urinary kallikrein activity and salt and water excretion in the rat. Am J Physiol 250:F1082–9.

Sengupta JN, Gebhart GF. 1994. Mechanosensitive properties of pelvic nerve afferent fibers innervating the urinary bladder of the rat. J Neurophysiol 72:2420–30.

Vaughan CW, Satchell PM. 1995. Urine storage mechanisms. Prog Neurobiol 46:215–37.

Vlaskovska M, Kasakov L, Rong W, et al. 2001. P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21:5670–7.

Watanabe T, Constantinou CE. 1996. Analysis of pressure/flow characteristics in the female rat and their pharmacologic modulation. Neurourol Urodyn 15:513–27.

Yarker YE, Goa KL, Fitton A. 1995. Oxybutynin. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in detrusor instability. Drug Ageing 6:243–62.

Yoshida M, Miyamae K, Iwashita H, et al. 2004. Management of detrusor dysfunction in the elderly: Changes in acetylcholine and adenosine triphosphate release during aging. Urology 63:17–23.

Yoshimura N, De Groat WC. 1997. Neural control of the lower urinary tract. Int J Urol 4:111–25.