INFLUENCE OF DICYCLOMINE ON IN VITRO REACTIVITY OF URINARY BLADDER SMOOTH MUSCLE IN GUINEA PIGS

MÁRIA JAKUBESOVÁ¹, HENRIETA RUSNÁKOVÁ¹, KATARÍNA PÁSZTÓOVÁ¹, JURAJ MOKRÝ¹, JÁN ŠVIHRA²

¹Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, ²Department of Urology, Jessenius Faculty of Medicine, Comenius University, Faculty Hospital of Martin, Martin, Slovakia

Abstract

Introduction: Nowadays, new therapeutic approaches are sought for treatment of functional disorders of urinary bladder, which could prevent the decrease of patients’ quality of life. One of the possibilities of conservative therapy is the administration of anticholinergics. The aim of this study was to verify the in vitro action of dicyclomine (used in the therapy of irritant bowel disease) on urinary bladder smooth muscle in guinea pigs and to compare its effect with previously tested oxybutynin.

Methods: The reactivity of the urinary bladder smooth muscle was estimated by in vitro method using organ chambers. The smooth muscle strips were prepared from guinea pig urinary bladders and aerated under the tension in Krebs-Henseleit’s solution in the organ bath. The cumulative concentration-response curves to acetylcholine (10⁻⁸-10⁻³ mol.l⁻¹) were plotted before and after adding of dicyclomine at concentration of 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ mol.l⁻¹.

Results: Dicyclomine caused decrease of urinary bladder smooth muscle reactivity to acetylcholine. This decrease was statistically significant only at the concentration of 10⁻⁴ and 10⁻³ mol.l⁻¹ of dicyclomine.

Conclusions: Dicyclomine significantly influenced the reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine. By comparing the influence of oxybutynin we can conclude, that oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine.

Key words: urinary bladder, contraction, oxybutynin, dicyclomine, smooth muscle

INTRODUCTION

An increasing prevalence of overactive bladder in the older population was described in various studies (1). Detrusor hyperactivity could be a result of several pathomechanisms, most probably with myogenic or neurological basis (2). Muscarinic receptors mediate normal bladder contraction, but also contractions of overactive bladder, so antimuscarinic drugs can block detrusor contractions in patients with bladder hyperactivity (3). There are also other ways to influence hyperreactivity, including drugs which have primary effects on membrane ion channels (Na⁺, Ca²⁺, K⁺), prostaglandin synthesis inhibitors (4) as well as agents modifying the activity of released mediators into the synaptic cleft. To sum up, also α-adrenoceptor antagonists, β-adrenoceptor agonists, vasopressin analogue, antidepressants like imipramine, botulotoxin, and capsaicin should be mentioned.

The antagonism of muscarinic receptors is the major mechanism of action of various drugs, used in many pathological conditions. Dicyclomine, as an agent used in the therapy of irritant bowel disease, seems to be usable (due to its potent antimuscarinic activity) also in overactive urinary bladder smooth muscle (5).

The aim of our study was to study the effects of dicyclomine on in vitro reactivity of urinary bladder smooth muscle in guinea pigs. Oxybutynin, which is able to decrease hyperactivity of detrusor by summation of effect of muscarinic receptors blockade and local anesthetic effect, is considered to be a conservative therapy standard of overactive urinary bladder in humans in Slovakia (6). In this study we compared its effect with that of dicyclomine.

METHODS

The reactivity of urinary bladder smooth muscle was estimated by in vitro method (7,8,9). 8 animals weighting 250-350 g were used. The preparations of urinary bladder smooth muscle

Address for correspondence:
Juraj Mokrý, MD., Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Sklabinská 26, SK-037 53 Martin, Slovakia
Phone: +421 43 4132535, fax: +421 43 4134807, e-mail: mokry@jfmed.uniba.sk
strips (2x2x15 mm) from guinea pigs were mounted between two hooks and placed into a 30 ml organ chamber containing Krebs-Henseleit buffer of the following composition: NaCl 110.00 mmol.l\(^{-1}\), KCl 4.80 mmol.l\(^{-1}\), CaCl\(_2\) 2.35 mmol.l\(^{-1}\), MgSO\(_4\) 1.20 mmol.l\(^{-1}\), KHPO\(_4\) 1.20 mmol.l\(^{-1}\), NaHCO\(_3\) 25.00 mmol.l\(^{-1}\) and glucose 10.00 mmol.l\(^{-1}\) in glass-distilled water. The organ chambers were maintained at 36.5 ± 0.5 °C and were aerated continuously with a mixture of 95% O\(_2\) and 5% CO\(_2\), to maintain pH 7.5 ± 0.1. One of the hooks was connected to a force transducer (TSR 10G, Vývoj Martin, Slovakia) and an amplifier (M1101 SUPR, Mikrotechna Praha, Czech Republic) and tension recordings were made on a Line Recorder TZ 4620 (Labotatoriumní přístroje Praha, Czech Republic). The tissue strips were initially set to 4 g of tension (30 minutes loading phase). After this period, the tension in each strip was readjusted to a baseline of 2 g (30 minutes adaptation phase). During both of the periods the tissue strips were washed at 10 minutes intervals. Thereafter, cumulative doses of acetylcholine (10\(^{-8}\) to 10\(^{-3}\) mol.l\(^{-1}\), subst. Sigma-Aldrich) were added and a continual graphical recording of contractions was made. This recording was named “Control”. After 25 minutes of washing-up period, water solution of dicyclomine (subst. Sigma-Aldrich) was added into each chamber in order to reach the concentrations of 10\(^{-6}\), 10\(^{-5}\), 10\(^{-4}\) and 10\(^{-3}\) mol.l\(^{-1}\). After 15 minutes period of incubation the amplitudes of contractions (g/100mg) of urinary bladder smooth muscle strips to the cumulative doses of acetylcholine (10\(^{-8}\) to 10\(^{-3}\) mol.l\(^{-1}\)) were recorded. These records were used for evaluation of the contractile responses (10).

A non-parametric ANOVA test was used for the statistical analysis. Results are presented as mean ± standard error of the mean (SEM). A probability level of p<0.05 was accepted as significant. All experiments were conducted in accordance with basic ethical norms and Helsinki Declaration of 1975, revised in 1983.

**RESULTS**

Addition of acetylcholine into the organ bath with urinary bladder smooth muscle strip in cumulative manner resulted in a dose-dependent increase of the contractile responses in controls. In the organ baths with dicyclomine in all of the concentrations, the contractile responses of urinary bladder smooth muscle were decreased. This decrease was statistically significant only at concentrations of 10\(^{-4}\) and 10\(^{-3}\) mol.l\(^{-1}\) of dicyclomine (Fig.1).

![Fig. 1](image-url) Reactivity of urinary bladder smooth muscle in guinea pigs after adding of dicyclomine to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) with standard error of the mean (SEM). One asterisk represents statistical significance of difference with p < 0.05 (DIC-6 = dicyclomine at concentration of 10\(^{-6}\) mol.l\(^{-1}\), etc.).
Figures 2 and 3 show the comparison of the urinary bladder smooth muscle reactivity to oxybutynin (6) and dicyclomine at concentration of $10^{-5}$ and $10^{-4}$ mol.l$^{-1}$ in guinea pigs. In both concentrations, oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine.

**Fig. 2** Comparison of the urinary bladder smooth muscle reactivity in guinea pigs after adding of dicyclomine (DIC, thick solid line) and oxybutynin (OXY, thin dashed line) in dose of $10^{-5}$ mol.l$^{-1}$ to cumulative doses of acetylcholine.

**Fig. 3** Comparison of the urinary bladder smooth muscle reactivity in guinea pigs after adding of dicyclomine (DIC, thick solid line) and oxybutynin (OXY, thin dashed line) in dose of $10^{-4}$ mol.l$^{-1}$ to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) with standard error of the mean (SEM). One asterisk represents statistical significance of difference with $p < 0.05$. 
DISCUSSION

The urinary bladder and any impairment of its functioning can influence the behavior of the whole organism, as the ability to accumulate urine and consecutively release it, belongs to basic social needs. Any changes in this basic need can disturb its integration and social positioning and so could lead to significant decrease of quality of life. Therefore, it is very necessary to study the mechanisms participating in the urinary bladder activity and to be able to modulate it in case of disorder.

The problems of hyperresponsiveness or hyperreactivity of smooth muscle in various organ systems, like respiratory system, gastrointestinal tract, and skin are found very often (11). Similarly, the urinary bladder „stability” problems are very often, too. Švíhra et al. (2001) showed in their recent study that overactive, „unstable”, bladder incidence in population of Slovakia rises especially with age (1). The frequent voiding and bladder fullness sensations, sensation of not complete bladder emptying after voiding and later also impaired ability to accumulate urine – incontinence – are considered as typical symptoms of overactive bladder. And especially the incontinence can significantly impair the patients’ quality of life.

The parasympathetic nervous system, similarly to other organ systems, plays the major role in the regulation of the urinary bladder smooth muscle (12). Five pharmacologically different muscarinic receptors (M₁-M₅) are distinguished, with representation of receptors M₂ a M₃ in humans. Although M₂ receptor was found to be the predominant one, the major role in contraction responses plays the M₃ receptor subtype (13,14).

The role of postsynaptic M₂ receptors is expressed as the ability to act against beta-adrenergic receptors, whose activation enables the urinary bladder smooth muscle relaxation and urine accumulation (15,16). Besides, M₂ receptor stimulation is associated with an activation of non-specific cation channels and inactivation of potassium channels (17,18). To conclude this, M₂ receptors are responsible for a direct contraction of the smooth muscle during voiding, until postsynaptic M₃ receptors prevent the relaxation of the smooth muscle evoked by sympathetic activation. These two effects are synergical in order to expel the urine from the urinary bladder effectively (19).

An abnormal stimulation of muscarinic receptors is responsible for the contractile properties of the urinary bladder smooth muscle in diseased state (overactive bladder). Muscarinic M₃ receptor antagonists have therapeutic potential for the treatment of disorders associated with altered smooth muscle contractility or tone. These include irritable bowel syndrome, chronic obstructive airways disease and urinary incontinence. Dicyclomine is a potent muscarinic receptor antagonist on the ileum with selectivity for M₃ receptor in absence of cardiovascular effects and with selectivity over inhibition of salivary secretion (20).

However, the musculotropic action of dicyclomine in bladder muscle has been attributed to its local anesthetic activity. This “local anesthetic” property is not sufficient to explain the action, as showed Downie and McGuire (21). They found that contractions elicited by replacement of calcium during depolarization with 80 mM K⁺ were only slightly affected by atropine or scopolamine, but were antagonized in a noncompetitive manner by dicyclomine (21).

Maggi and Meli (5) showed that intravenously administered dicyclomine produced a dose-dependent decrease of eserine-induced muscle tone and suppressed phasic contractions in rabbit detrusor (5).

In our in vitro experiments using organ baths we found that dicyclomine causes dose dependent decrease of acetylcholine-induced contractions in guinea pigs urinary bladder smooth muscle. This finding is in consistence with literary sources regarding the effect of dicyclomine on gastrointestinal smooth muscle.

One of the most effective treatments for overactive bladder disease represents the anticholinergic therapy (oxybutynin) (22). Oxybutynin causes depression of detrusor hyperactivity, which is reached by the blockade of muscarinic receptors, direct relaxation of detrusor and by a local anesthetic effect. However, the local anesthetic effect is present only by
intravesical administration of oxybutynin. Oxybutynin possesses higher affinity to muscarinic receptors M1 and M3 than to M2 subtype. The clinical importance of this affinity is still unclear, as oxybutynin acts through its active metabolites. Oxybutynin solidly inhibits the urinary bladder hyperreactivity and is therefore recommended as first line therapy of the overactive bladder (22).

However, the dryness in mouth during a standard dosage regimen was reported in a relatively high number of patients – 80 % (22). Therefore, other agents with potential effect on the urinary bladder smooth muscle are tested, including calcium channel blockers verapamil and nifedipine (23), highly selective antagonist of M₃ receptor subtype darifenacine (24), or imipramine with anticholinergic action and 5-hydroxytryptamine blocking effect (22). Local effect and desensitization of the sensory receptors in urinary bladder participates in effect of intravesically-administered capsaicin (25).

Dicyclomine, used in the therapy of irritant bowel disease, showed in our in vitro experiments the ability to suppress the contractility of urinary bladder smooth muscle, which was, however, significantly lower than that of oxybutynin in the same concentration. Therefore, its usage in urinary bladder disorders is questionable and needs further studies.

In conclusion, according to our results, we can confirm that dicyclomine significantly influenced the reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine. By comparing the influence of oxybutynin (6) we can conclude that oxybutynin caused significantly stronger decrease of reactivity to acetylcholine than dicyclomine. These findings are still objects of further research.

REFERENCES

This work was supported by Grant APVT-20-0131-02, and Comenius University Grants No. 212/2003/UK and 35/2004/UK and presented at Student Scientific Conference, Martin, 2004

Received: July, 12, 2004
Accepted: October, 18, 2004