EXPERIMENTAL STUDY

Propantheline and in vitro reactivity of urinary bladder smooth muscle in guinea pigs

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Abstract

Objective: **The aim of this study was to verify the in vitro action of propantheline on urinary bladder smooth muscle in guinea pigs and to compare its effect with previously tested oxybutynin.**
Materials and methods: **The reactivity of the urinary bladder smooth muscle was estimated in vitro 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 constructed before and after 15 minute incubation with propantheline in concentration of 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} mol.l^{-1}.**
Results: **Propantheline caused a decrease of urinary bladder smooth muscle reactivity to acetylcholine. This decrease was statistically significant only at concentration of 10^{-5}, 10^{-4} and 10^{-3} mol.l^{-1} of propantheline.**
Conclusions: **Propantheline significantly influenced the reactivity of urinary bladder smooth muscle to acetylcholine in guinea pigs. Comparing the influence of oxybutynin we can conclude that oxybutynin caused a significantly higher decrease of the reactivity to acetylcholine than propantheline.** ***(Fig. 3, Ref. 30.)***
Key words: urinary bladder, contraction, oxybutynin, propantheline, smooth muscle.

In various studies, an increasing prevalence of overactive bladder in the older population was described (1, 2). There are several pathomechanisms, most probably on myogenic or neurological basis, underlying the detrusor hyperactivity as well as urinary incontinence (3, 4, 5). Muscarinic receptors mediate normal bladder contraction, but also contractions of overactive bladder, so antimuscarinic drugs can block detrusor contractions in patients with bladder hyperactivity (6). There are also other ways of pharmacological influencing the hyperreactivity and incontinence, including drugs with primary effects on membrane ion channels (Na^+, Ca^{2+}, K^+), prostaglandin synthesis inhibitors (7) as well as agents modifying the activity of released mediators into the synaptic cleft. Furthermore, α-adenoceptor antagonists and agonists, ß-adrenoceptor agonists and antagonists, vasopresin analogue, antidepressants like imipramine, botulotoxin, estrogenes, and capsaicin should be mentioned (8, 9).

The antagonism of muscarinic receptors is the major mechanism of action of various drugs, used in many pathological conditions. Propantheline, an agent used in the therapy of irritant bowel disease, a supportive measure for patients receiving etoposide regimens (10), in preventing neurocardiogenic syncope (11), a good therapeutic choice in spinal cord injured patients with excessive sweating and neurogenic bladder dysfunction (12), is useful (due to its potent antimuscarinic activity) also in overactive urinary bladder smooth muscle (13).

The aim of our study was to study the effects of propantheline on in vitro reactivity of urinary bladder smooth muscle in guinea pigs. Standard conservative therapy of overactive urinary bladder in humans in Slovakia is oxybutynin, which is able to decrease hyperactivity of detrusor by summation of the effect of muscarinic receptors blockade and local anesthetic effect (14). In this study we compared the effect of oxybutynin with propantheline.

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Methods

The reactivity of urinary bladder smooth muscle was estimated by in vitro method (15, 16, 17). Eight animals, weight 250–350 g, were used. The preparations of urinary bladder smooth muscle 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 7.54±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 (Labotatorní 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 periods were the tissue strips washed at 10 minute 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 propantheline (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 minute incubation were amplitudes of contractions (g/100 mg) of urinary bladder smooth muscle strips under cumulative doses of acetylcholine (10^{-8} to 10^{-3} mol.l^{-1}) recorded. These records were used for evaluation of contractile responses (14).

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 from 1975, revised in 1983.

Results

Addition of acetylcholine into the organ bath with urinary bladder smooth muscle strip in cumulative manner resulted in dose-dependent increase of the contractile responses in controls. In the organ baths with propantheline, in all concentrations, the contractile responses of urinary bladder smooth muscle decreased. This decrease was statistically significant in concentrations 10^{-5}, 10^{-4} and 10^{-3} mol.l^{-1} of propantheline (Fig. 1).

Figures 2 and 3 show the comparison of the urinary bladder smooth muscle reactivity to oxybutynin (14) and propantheline at concentration of 10^{-5} and 10^{-4} mol.l^{-1} in guinea pigs. At both concentrations, oxybutynin caused a significantly higher decrease of the reactivity to acetylcholine than propantheline.

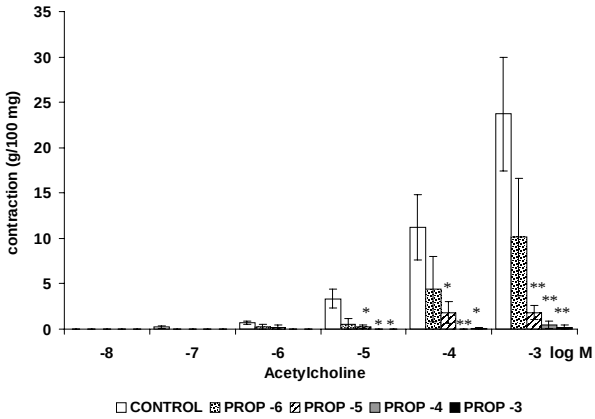

Fig. 1. Reactivity of guinea pig urinary bladder smooth muscle after adding propantheline to cumulative doses of acetylcholine. The columns represent a mean contraction (g/100 mg) and visible range represents a standard error of the mean (SEM). One, or two asterisks, represent a statistical significance of difference $p<0.05$, and $p<0.01$, respectively (PROP-6 = propantheline in concentration of 10^{-6} mol.l^{-1}, etc.).

Discussion

The urinary bladder or any impairment of its function can influence the behavior, as the ability to accumulate urine and release it consecutively belongs to basic social needs. Any changes in this basic need can disturb the integration and social position and could lead to a significant decrease of quality of life. Therefore it is necessary to study the mechanisms participating in the urinary bladder activity and to modulate it when needed.

The problem of hyperresponsiveness or hyperreactivity of smooth muscle in various organ systems, like respiratory system, gastrointestinal tract, and skin are very frequent (15). Similarly, the urinary bladder "stability" problems are very frequent, too. Švihra et al (2001) showed, in a recent study that overactive, "unstable", bladder incidence in population of Slovakia raises 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. Especially the incontinence can significantly impair the patients` quality of life (18).

The parasympathetic nervous system, similarly in other organ systems, plays the major role in the regulation of the urinary bladder smooth muscle (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. Propantheline is a potent muscarinic receptor antagonist of the smooth muscle in gastrointestinal tract (20), as

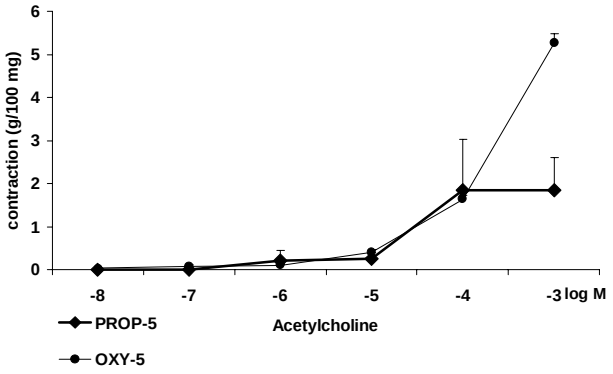

Fig. 2. Comparison of the guinea pig urinary bladder smooth muscle reactivity after adding propantheline (PROP, thick line) and oxybutynin (OXY, thin line) at concentration 10^{-5} mol.l^{-1} to cumulative doses of acetylcholine.

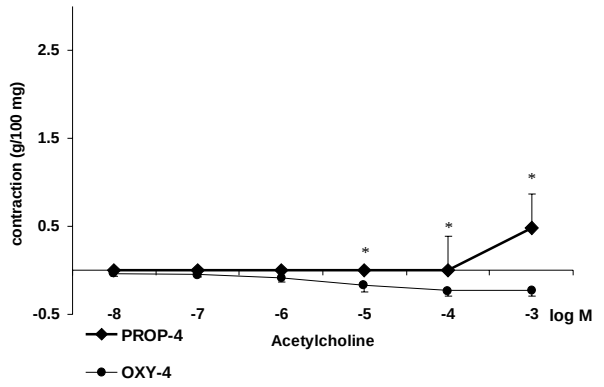

Fig. 3. Comparison of the guinea pig urinary bladder smooth muscle reactivity after adding propantheline (PROP, thick line) and oxybutynin (OXY, thin line) at concentration 10^{-4} mol.l^{-1} to cumulative doses of acetylcholine. One asterisk represents a statistical significance of difference $p<0.05$.

well in urinary bladder (21), with selectivity for M3 receptor. Like other quaternary ammonium compounds, propantheline is incompletely absorbed following oral administration. It is extensively metabolized in the gut and liver, principally to xanthanoic acid and its glucuronide and diisopropylmethyl ethanolamine, and to a lesser extent, to hydroxypropantheline and hydroxyxanthanoic acid and its glucuronide (22). Many interactions have been described, especially due to its inhibitory effect on gastrointestinal tract motion and liver metabolism (23).

In unanesthetized rats Ukimura (1993) showed that after intravesical administration propantheline causes a remarkable suppression of maximum intravesical pressure accompanied with a consistent increase of residual rate and bladder capacity in a dose-dependent manner (24).

Guarneri et al (1993) studied the effects of drugs used in the therapy of detrusor hyperactivity on volume-induced contractions of the rat urinary bladder in vivo. They found that there is a possibility of distinguishing antimuscarinics (propantheline, emepronium, oxybutynin) and calcium antagonists (nifedipine, terodiline), which decrease the amplitude of the voiding contractions in dose dependent way peripherally after intravenous administration, and other drugs, inducing a decrease in the frequency of the voiding acting on the micturition center(s) in the central nervous system (flavoxate, prazosin, indomethacin) (25).

We found in in vitro experiments using organ baths that propantheline causes dose-dependent decrease of acetylcholine induced contractions in guinea pigs urinary bladder smooth muscle. This finding is in consistence with literature sources.

One of the most effective treatments of overactive bladder disease is the anticholinergic therapy (oxybutynin) (26). Oxybutynin causes depression of detrusor hyperactivity, which is reached by the blockade of muscarinic receptors, direct relaxation of detrusor and by local anesthetic effect. However, the local anesthetic effect is present only in intravesical administration of oxybutynin. Oxybutynin possesses a higher affinity to muscarinic receptors M1 and M3 than 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 (13, 26, 27).

However, the dry mouth during standard dosage regimen was reported in a relatively high number of patients – 80 % (26). Thuroff et al (1991) showed that oxybutynin has statistically significant effects on subjective symptoms and objective urodynamic parameters in patients with detrusor hyperactivity compared to propantheline. Also in this study, dry mouth was the major complaint. The rate of inquired adverse effects was significantly higher for oxybutynin (58.2 %) versus propantheline (44.7 %) and placebo (43.4 %) (28). Therefore other agents with potential effect on the urinary bladder smooth muscle are tested, including calcium channel blockers verapamil and nifedipine, highly selective antagonist of M3 receptor subtype darifenacine (29), or imipramine, with anticholinergic action and 5-hydroxytryptamine blocking effect (26). Local effect and desensitization of the sensory receptors in urinary bladder participates in the effect of intravesically-administered capsaicin (30).

Anticholinergic agents should be the first line pharmacological therapy for patients with detrusor instability. Oxybutynin is the anticholinergic of choice, whereas propantheline is the second-line therapy. Although calcium antagonists have been studied, drug introduced for the treatment of overactive bladder – terodiline – was withdrawn from the market due to a risk of cardiac arrhythmia. Studies of potassium channel openers have found either lack of clinical efficacy or an unacceptable level of side effects. Alpha-adrenergic antagonists may be useful for decreasing bladder overactivity in patients with autonomous bladders as a result of spinal cord injury. Tricyclic antidepressants – imipramine – may also be effective in decreasing bladder contractility (13).

In conclusion, based on our results, we can confirm that propantheline significantly influenced the reactivity of urinary bladder smooth muscle to acetylcholine in guinea pigs. Comparing the influence of oxybutynin (14) we can conclude, that

oxybutynin caused a significantly higher decrease of the reactivity to acetylcholine than propantheline. These findings are objects of further research.

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