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EXPERIMENTAL STUDY

In vitro reactivity of urinary bladder smooth muscle in rabbits influenced by xanthine derivatives

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Abstract: *Introduction:* The contractions of urinary bladder smooth muscle are evoked by parasympathetic nervous system, with its main mediator acetylcholine. These contractions can be inhibited by two basic mechanisms – inhibition of contraction (anticholinergic drugs) or inducing the relaxation (sympathomimetics, calcium channel blockers). In this study, we investigated the effect of caffeine and theophylline – both are non-selective inhibitors of phosphodiesterase – on urinary bladder smooth muscle contractions evoked by acetylcholine.
Methods: The reactivity of the urinary bladder smooth muscle was estimated by in vitro method using organ chambers.
Results: Caffeine and theophylline caused decrease of urinary bladder smooth muscle reactivity to acetylcholine. This decrease was statistically significant only in concentrations of 10^{-4} and 10^{-3} mol.l^{-1} of caffeine and theophylline.
Conclusions: Caffeine and theophylline significantly influenced the reactivity of urinary bladder smooth muscle in guinea pigs to acetylcholine. By comparing the influence of aminophylline we can conclude, that caffeine as well as theophylline caused significantly stronger decrease of the reactivity to acetylcholine than aminophylline only in lower concentration *(Fig. 4, Ref. 30).* Full Text (Free, PDF) www.bmj.sk.
Key words: urinary bladder, contraction, caffeine, theophylline, aminophylline, smooth muscle.

In several studies, an increasing prevalence of overactive bladder in the older population was described (1, 2). Overactive bladder is a condition resulting from involuntary contraction of the smooth muscle in the wall of the urinary bladder. It causes a sudden and unstoppable need to urinate (urinary urgency). The most probable pathomechanisms are based on myogenic or neurological origin, leading to 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. However, this therapy is associated with relatively high incidence of adverse effects (6). For example, the dry mouth during a standard dosage regimen was reported in a relatively high number of patients – 80 % (7). Thus, different mechanisms of action are widely discussed and other ways of pharmacological management of the hyperreactivity and incontinence are evaluated (8). These include drugs with primary effects on membrane ion channels (Na^+, Ca^{2+}, K^+), prostaglandin synthesis inhibitors, agents modifying the activity of released mediators into the synaptic cleft, α-adrenoceptor antagonists, β-adrenoceptor agonists, vasopresin analogues, antidepressants like imipramine, botulotoxin, as well as estrogens (9, 10). Furthermore, local effect and desensitization of the sensory receptors in urinary bladder participates in the effect of intravesically-administered capsaicin, which increases the storing capacity of bladder and decreases incontinence (3). In several therapeutic choices, decreased cytoplasmatic levels of calcium or increased levels of cyclic AMP are considered to be the crucial steps in affecting contraction. Thus, inhibitors of the enzyme phosphodiesterase, which is responsible for degradation of cyclic AMP, might be of benefit in bladder overactivity.

There are many pharmacological agents, which can inhibit phosphodiesterase – either selectively or non-selectively. Among them, especially xanthine derivatives (methylxanthines) are used often in the therapy of chronic inflammatory diseases associated with increased airway smooth muscle reactivity (11). However, except of increasing cAMP levels in smooth muscle, we cannot omit other positive mechanisms, potentially enforced in their action. Among them, competitive antagonism of adenosin receptors (12) as well as their central action on A1 and A2 receptors (purinergic P1 receptor superfamily), anti-inflammatory effects (potentially usefull in overactivity associated with interstitial cystitis), membrane hyperpolarization due to opening the

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potassium channels, effects on intracellular calcium stores and its release, and changed secretion or activity of endogene mediators are to be mentioned (13, 14, 15). Therefore, inhibitors of phosphodiesterease with selectivity to isoenzymes with localization in urinary bladder smooth muscle would be of greatest importance (16). This inhibition could lead to increased levels of cAMP and detrusor relaxation as well as to potentiation of β agonists, effect (17). From these, especially isoenzymes PDE III and IV were already studied. Gillespie (18) found that zardaverine (PDE III and IV inhibitor) and Ro 20-1724 (PDE IV inhibitor) led to most potent decrease of frequency and amplitude of phasic activity in isolated guinea pig urinary bladder.

Previously we found that aminophylline is able to suppress the contractile responses of rabbit urinary bladder smooth muscle to acetylcholine (19). In order to support these results and to extend them to other representatives of xanthine derivatives, the aim of the presented study was to evaluate the effects of caffeine and theophylline on in vitro reactivity of urinary bladder smooth muscle in healthy rabbits.

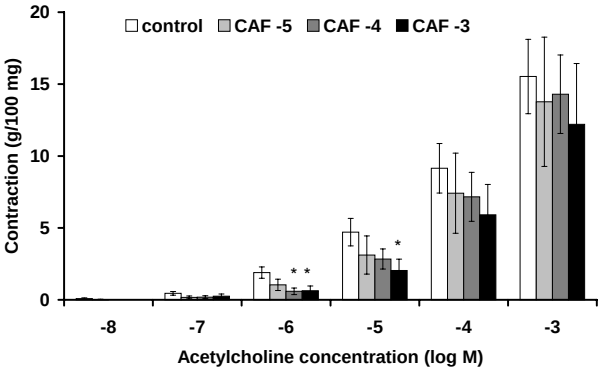

Fig. 1. Influence of caffeine on the reactivity of rabbit urinary bladder smooth muscle to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and showed range represents standard error of the mean (SEM). One asterisk represents statistical significance of difference with p<0.05 (CAF-5 = caffeine in concentration of 10^{-5} mol.l^{-1}, etc.).

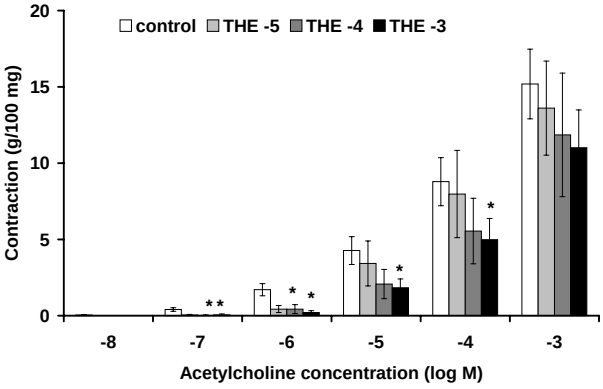

Fig. 2. Influence of theophylline on the reactivity of rabbit urinary bladder smooth muscle to cumulative doses of acetylcholine. The columns represent mean contraction (g/100 mg) and showed range represents standard error of the mean (SEM). One asterisk represents statistical significance of difference with p<0.05 (THE-5 = theophylline in concentration of 10^{-5} mol.l^{-1}, etc.).

Methods

The reactivity of urinary bladder smooth muscle in rabbits was estimated by in vitro method (19, 20). 8 animals weighting 2900–4100 g were used. The preparations of urinary bladder smooth muscle strips (2x2x15 mm) 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 10 G, 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 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 caffeine (subst. Sigma-Aldrich) or theophylline (subst. Sigma-Aldrich) was added into each chamber in order to get the concentrations of 10^{-5}, 10^{-4} and 10^{-3} mol.l^{-1}. After 15 minutes period of incubation the amplitudes of contractions (g/100 mg) 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 (21, 22).

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 approved by local ethics committee and conducted in accordance with basic ethical principles and Helsinki Declaration of 1975, revised in 1983.

Results

The 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 caffeine in all of the concentrations, the contractile responses of urinary bladder smooth muscle were decreased. This decrease was statistically significant for concentrations of 10^{-4} and 10^{-3} mol.l^{-1} of caffeine (Fig. 1).

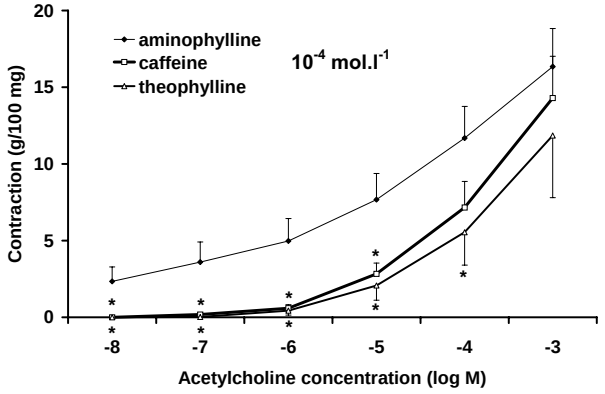

Fig. 3. Comparison of the rabbit urinary bladder smooth muscle reactivity to cumulative doses of acetylcholine in presence of caffeine, theophylline, and aminophylline at concentration of 10^{-4} mol.l^{-1}. One asterisk represents statistical significance of difference with $p<0.05$.

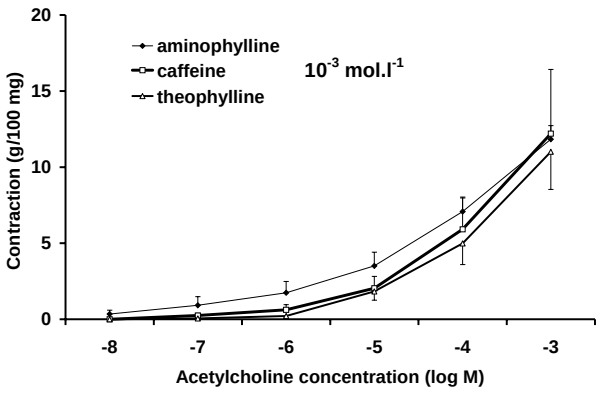

Fig. 4. Comparison of the rabbit urinary bladder smooth muscle reactivity to cumulative doses of acetylcholine in presence of caffeine, theophylline, and aminophylline at concetration of 10^{-3} mol.l^{-1}.

The addition of theophylline into the organ baths caused a decrease of the contractile responses of urinary bladder smooth muscle in all concentrations, with significance observed only for concentrations of 10^{-4} and 10^{-3} mol.l^{-1} of theophylline (Fig. 2).

There was no significant difference between the action of caffeine and theophylline on decreasing the urinary bladder smooth muscle reactivity. Figures 3 and 4 show the comparison of the urinary bladder smooth muscle reactivity to aminophylline (19) with that of caffeine and theophylline in concentration of 10^{-4} and 10^{-3} mol.l^{-1} in rabbits. In both concentrations, caffeine and theophylline caused stronger decrease of reactivity to acetylcholine than aminophylline. However, this decrease was statistically significant only in lower concentration (10^{-4} mol.l^{-1}).

Discussion

The contractile response in urinary bladder smooth muscle can be modulated in principle by two different mechanisms: 1) via inhibiting the contraction through membrane receptors – e.g. competitive antagonism of muscarinic receptors (anticholinergics), influencing purinergic and vanilloid receptors as well as agonistic action on beta receptors and 2) via inducing relaxation through affecting the intracellular levels of second messengers – e.g. inhibition of phosphodiesterase (xanthine derivatives), as well as changing the membrane potential and thus stabilizing the membrane of smooth muscle cells, like decreasing the ionised calcium levels (calcium channel blockers), opening potassium channels, etc. Both of these mechanisms are exerted at the level of nervous synapses or smooth muscle cells (23,2 4). Nevertheless, some agents can act on both levels (β2 mimetics).

Currently, the pharmacological modulation of overactive bladder includes especially the drugs acting at the level of muscarinic receptors, membrane ion channels, adrenergic receptors, antidepressants with central action, as well as agents with mixed effects (7).

For our experiments, we chose theophylline and caffeine as representatives of xanthine derivates routinely used (theophylline) in clinical practice for the treatment of obstructive diseases of airways (asthma, COPD) (25). Pharmacodynamic properties of methylxanthines are based on their multi-organ effects (26, 27). Among them, stimulation of central nervous system leading to positive stimulation of breathing, tremor, convulsions, anxiety or vasoconstriction should be mentioned. Furthermore, in cardiovascular system their administration leads to an increase of cardiac output, decrease of heart fibrillation and dilatation of peripheral arteries and veins. They increase blood flow through the kidneys, glomerular filtration and thus also diuresis. Especially this effect could be a limiting factor in potential therapeutic ambitions of xanthines, as increased diuresis could enhance the incontinence rate in individuals with clinical predisposition. Except for that, antiinflammatory action and relaxation of smooth muscle (bronchial, uterine, and gastrointestinal) are of importance (28).

In our previous experiments (19), we showed potential benefit of aminophylline in the modulation of urinary bladder smooth muscle reactivity to acetylcholine. However, the significant relaxing effect was observed only in relatively high in vitro concentration of aminophylline, which cannot be achieved in organism under clinical conditions without adverse effects. Therefore, in this study we evaluated the in vitro efficacy of other methylxanthines – caffeine and theophylline – in suppression of contractile responses to acetylcholine.

Presented results supported our hypothesis that inhibition of phosphodiesterase can suppress the contractile responses to acetylcholine. However, we cannot exclude other mentioned mechanisms, which could influence this relaxation.

Our results are supported by older data of Dangor and Johns (29) who demonstrated significant decrease of urinary bladder smooth muscle reactivity to histamine, electric field stimulation and ATP at theophylline concentration of 10^{-3} mol.l^{-1}. However,

they did not find any significant influence of theophylline in lower concentrations (10^{-5} and 10^{-4} mol.l^{-1}).

Contrary, Lee et al (30) showed that caffeine in low concentration ($0.4.10^{-6}$ mol.l^{-1}) significantly increased the frequency of detrusor contractions. However, in their study lower concentrations of extracellular calcium and electric stimulation were used.

Our results showed that nonselective inhibitors of phosphodiesterase caffeine and theophylline are relatively effective inhibitors of contractile responses of urinary bladder smooth muscle. Their effect was even higher comparing to aminophylline (complex of theophylline and ethylenediamine). However, from clinical point of view, the concentrations of both caffeine and theophylline with significant suppression were relatively high, as recommended serum concentration of theophylline is 8–15 μg.ml^{-1}. Crossing these limits can lead to severe adverse effects (19, 14).

In conclusion, theophylline and caffeine showed to be effective inhibitors of urinary bladder smooth muscle contractions in rabbits evoked by acetylcholine in vitro. Their effectiveness was even higher comparing to aminophylline. However, other inhibitors of phosphodiesterase (especially selective ones) have to be tested and wider spectrum of concentrations to be used to get better aspect on their possible effect on urinary bladder smooth muscle in healthy and diseased state and on their possible future therapeutic use.

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