

EFFECTS OF SPASMOLYTIC DRUGS AND EXPRESSION OF MUSCARINIC AND PURINERGIC RECEPTORS IN HUMAN DETRUSOR MUSCLE FROM DIFFERENT AGE GROUPS

Aims of Study

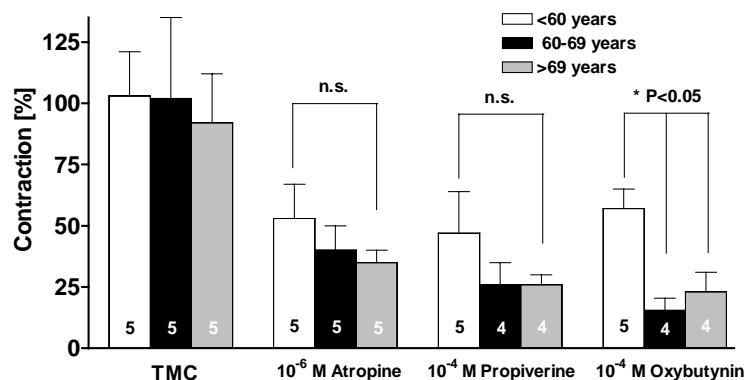
Cholinergic and purinergic neurotransmission in strips of human detrusor muscle have been reported to depend on the age of the patient [1]. Here we have investigated the effects of atropine and the spasmolytic drugs propiverine and oxybutynin in urinary bladder muscle using contractile responses to electric field stimulation (EFS) as an indicator of neurotransmission. The expression of the muscarinic receptors M₂ and M₃ and the purinoceptors P2X₁ and P2X₃ was measured in these and additional tissue samples. The aim of our study was to find out whether drug responses and receptor expression in detrusor muscle are related to age.

Methods

Tissue samples of the human urinary bladder were obtained from patients undergoing total cystectomy because of bladder carcinoma. All patients gave informed consent. The study was approved by the local Hospital Ethics Committee. Detrusor strips of 35 patients (28 male, 7 female; age: 68 ± 2 years; range: 43 to 84 years) were taken from tissue with no macroscopic sign of tumor. The serosa and mucosa layers were removed and samples were immediately frozen in fluid nitrogen for RT-PCR studies. Four to 6 muscle strips were dissected for contraction studies. The contractility of the tissue was measured in 25-ml organ baths (carbogen-gassed Tyrode's solution; 37°C) using isometric force transducers. The effects of atropine, propiverine and oxybutynin on the electric field stimulation (EFS; frequency of 30 Hz; pulses of 1 ms; train duration of 2 s with 2 min intervals between two stimulations) were studied in comparison to experiments without any drug added (time-matched controls, TMC). Cumulative concentration-response curves (CRC) for carbachol (10⁻⁸ M to 10⁻³ M) were also generated. For semiquantitative RT-PCR, total RNA was isolated from the frozen tissue and reverse transcribed with primer pairs specific for the muscarinic receptors M₂ and M₃ and the purinoceptors P2X₁ and P2X₃. The amplicons were normalized to calponin as housekeeping gene. The results of individual preparations were pooled into 3 age groups (see Fig. 1). Statistical significance between groups was analysed by one-way ANOVA or unpaired Student's t-Test.

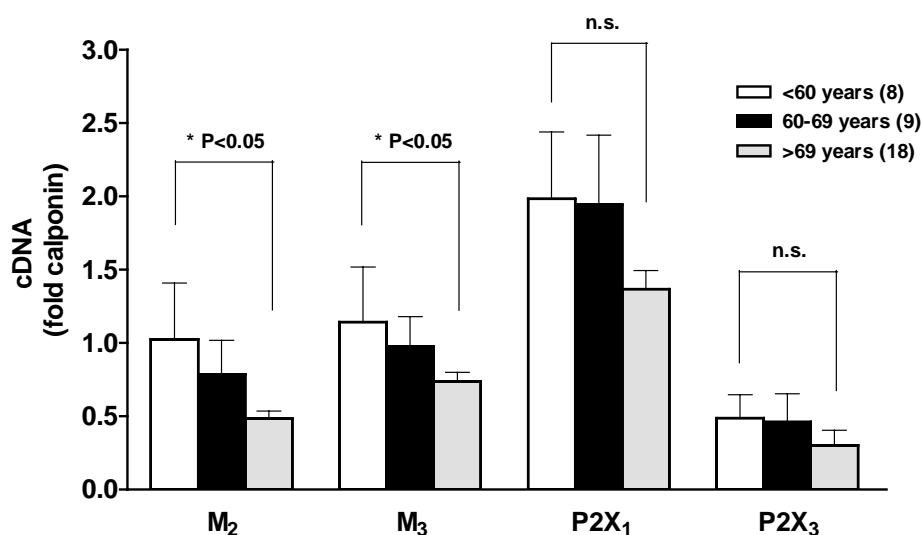
Results

Figure 1 – Comparison of the effect of atropine, propiverine and oxybutynin on the electrically stimulated contraction in detrusor muscle strips in different age groups compared to time-matched control (TMC) experiments (mean ± S.E.M.; pre-drug control = 100 %)



In human detrusor muscle strips electric field stimulation (EFS) evoked contraction amplitudes of large variation. When corrected for the wet weight of the strips, force of contraction did not depend on the age of the patient. Next, we tested whether there were any age-dependent differences in responses to the inhibitory effects of the spasmolytic drugs atropine, propiverine and oxybutynin on EFS-evoked contractions. No systematic change in drug sensitivity with increasing age was observed, i. e. the calculated $-\log IC_{50}$ values for the three spasmolytics were similar in each age group. However, the maximum response appeared to increase with age. Atropine (10^{-6} M) reduced EFS-evoked contractions to 52 ± 14 %, 40 ± 10 % and 35 ± 5 % of pre-drug control in the three age groups (Fig. 1), although, these differences did not reach the level of statistical significance. Similar results were obtained with propiverine, whereas oxybutynin was significantly more effective in the older patients (Fig. 1). We also analysed CRC for the muscarinic agonist carbachol and the shifts induced by the spasmolytic drugs on these curves. No age-dependent effects were detected. The semiquantitative RT-PCR studies showed that the expression of the muscarinic receptors M_2 and M_3 was significantly reduced with increasing age (Fig. 2). In addition, the expression of the purinoceptor $P2X_1$ was lower at higher ages, although the level of significance was not reached.

Figure 2 – Comparison of the expression of M_2 , M_3 , $P2X_1$ and $P2X_3$ receptors in the detrusor muscle of patients in different age groups (mean \pm S.E.M.). cDNA was normalized to the expression of the smooth muscle protein calponin.



Conclusions

In the tissue samples studied we found a tendency of increasing effectiveness of inhibition of EFS-evoked force of contraction by spasmolytic drugs with increasing age of the patients, whereas expression of muscarinic receptor subtypes M_2 and M_3 at the mRNA level was significantly reduced in older patients. Although we are well aware of the fact that mRNA levels do not allow conclusions about functional receptors, the findings of a larger suppression of EFS-evoked contraction by the investigated spasmolytics in the higher age groups in combination with lower receptor expression was unexpected. We suggest that these drugs may have other mechanisms of action in addition to antimuscarinic effects, which are differently regulated during aging.

References

[1] M. Yoshida, Y. Homma, A. Inoue, M. Yono, H. Seshita, Y. Miyamoto, S. Murakami, K. Kawabe, S. Ueda; 2001. Age-related changes in cholinergic and purinergic neurotransmission in human isolated bladder smooth muscles. *Exp. Gerontol.* 36: 99-109.