

DIRECT QUANTIFICATION OF MUSCARINIC RECEPTOR SUBTYPES IN HUMAN DETRUSOR MUSCLE, MUCOSA AND PAROTID GLAND BY IRREVERSIBLE INACTIVATION OF M₃ RECEPTORS BY 4-DAMP MUSTARD

Hypothesis / aims of study

Muscarinic receptors are expressed in the bladder mucosa as well as the detrusor muscle [1], which contribute to the micturition function. It is generally accepted that M₃ subtype plays a major role in the contraction of the detrusor muscle. It is reported that there are mRNA expressions for all muscarinic receptor subtypes in the detrusor and bladder mucosa of human, and that M₂ subtype is dominant [2]. However, direct information of relative distribution of pharmacologically relevant muscarinic receptor subtypes is still lacking. The alkylation of M₃ receptors by 4-DAMP mustard, leading to the irreversible inactivation of this subtype, was developed by Ehlert et al. [3]. This method is considered as a powerful way to quantify comparatively the pharmacologically relevant muscarinic receptor subtype in different tissues. The present study was conducted to compare the distribution of muscarinic receptor subtypes in the detrusor muscle, bladder mucosa and parotid gland of humans by the pretreatment with 4-DAMP mustard.

Study design, materials and methods

Specimens of human bladder were collected from bladder carcinoma patients (5-7 person) undergoing the open surgery. Specimens of human parotid gland were obtained from patients with the surgical excision of parotid tumor (8 person). Specimens were taken from macroscopically normal areas of the bladder. The bladder segment was dissected into detrusor and mucosa (urothelium and lamina propria), and stored at -80°C until use. In experiments where 4-DAMP mustard was used, the compound was first incubated at 37°C for 30 min in KRB buffer, pH7.4, to allow formation of the reactive aziridinium ion (cyclized 4-DAMP mustard). At first, the tissue homogenate was incubated with AF-DX116 to block M₂ receptor reversibly and then added cyclized 4-DAMP mustard to block M₃ receptor irreversibly. Finally, the reaction was stopped by sodium thiosulfate. The muscarinic receptor in tissue homogenates was measured by radioreceptor binding assay with [N-methyl-³H]scopolamine ([³H]NMS) as a radioligand, and binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [³H]NMS were estimated by Scatchard analysis. Then we compared the B_{max} for specific [³H]NMS binding in human tissues without and with 4-DAMP mustard pretreatment.

Results

Specific binding of [³H]NMS (0.1 to 0.5 nM) in homogenates of human detrusor muscle, bladder mucosa and parotid gland increased the concentration-dependently, and it appeared to be saturable around 1 nM. The 4-DAMP mustard treatment had little significant effect on K_d values for specific binding of [³H]NMS in these tissues. On the other hand, the B_{max} values for specific binding of [³H]NMS were significantly (36%, 41% and 63%, respectively) decreased in the detrusor, bladder mucosa and parotid gland treated with 4-DAMP mustard, compared to those without this treatment (Fig. 1).

Interpretation of results

The major findings of this study are that 1) there exist significant amounts of pharmacologically relevant M₂ and M₃ subtypes in the detrusor muscle, bladder mucosa and parotid gland of humans, and 2) M₂ subtype is predominant in the detrusor muscle and mucosa while M₃ subtype is dominant in the parotid gland.

Concluding message

The present study using 4-DAMP mustard has directly demonstrated that pharmacologically relevant M₂ and M₃ receptor subtypes coexist at the significant level in the detrusor muscle, bladder mucosa and parotid gland of humans with the different ratios. Thus, we believe that this work will contribute significantly to the further understanding of therapeutic effects of anticholinergic agents currently used to treat overactive bladder.

References

- [1] J Urol (2006) 176; 1673-1678.
- [2] Br J Pharmacol (2005) 144; 1089-1099.
- [3] J Pharmacol Exp Ther (2005) 313; 368-378.

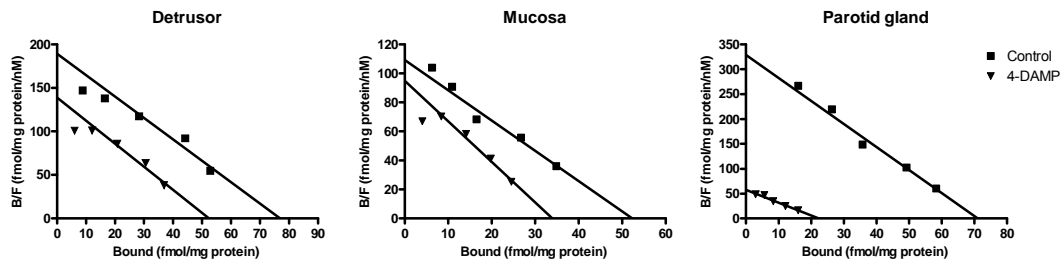


Fig. 1. Scatchard plots for significant decrease of maximal binding sites (Bmax) of [³H]NMS in the human detrusor muscle, mucosa and parotid gland without (control, ■) and with (▼) the pre-treatment with 4-DAMP mustard.

FUNDING: Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan

HUMAN SUBJECTS: This study was approved by the University of Shizuoka, University of Yamanashi, Hamamatsu University School of Medicine and followed the Declaration of Helsinki Informed consent was obtained from the patients.