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IN VIVO IMAGING ANALYSIS OF THE URINARY FUNCTION IN M3 MUSCARINIC RECEPTOR KNOCKOUT MICE

Hypothesis / aims of study

In the detrusor smooth muscles, M3 muscarinic acetylcholine receptor is known to play dominant roles in eliciting detrusor contraction. Using the M3 muscarinic receptors knockout (M3KO) mice, we found that M3 receptors mediate 95% of the cholinergic contraction of the detrusor muscle in both sexes in vitro and that lack of the M3 receptor resulted in severe urinary retention in males in vivo (Figure 1). However, to our knowledge, the natural course of postvoid residual urine (PVR) in the M3KO mice has not been investigated in special reference to such gender differences. The purpose of this study is to clarify the role of M3 receptor in conscious urinary function in both sexes and to search for the possible compensatory mechanisms of urinary function in lacking of M3 mechanism.

Study design, materials and methods

The PVR and micturition volumes of wild type (WT) and M3KO conscious mice of both sexes were measured from 5 to 10 weeks old. The PVR immediately after the monitored urination was measured by maximum section planimetry acquired by a small diameter (6 Fr) miniature transrectal ultrasonography (TRUS), using the semiplanimetric ellipsoid formula. Age-related changes in the urinary tract of M3KO were studied using elderly (1.5 year old) mice of both sexes using TRUS and magnetic resonance imaging (MRI). In order to compare the responses of the urinary function after subcutaneous administrations of (a) saline, (b) atropine (ATR, 1 mg/kg, s.c.) (muscarinic receptor antagonist) or (c) sodium salicylate (SS, 200 mg/kg, s.c.)(cyclo-oxigenase inhibitor), the PVR after the administration were measured using 10 – 12 week-old M3KO and wild type (WT) mice of both sexes.

Results

In males, the PVRs of the M3KO mice were greater than those of age-matched WT during 5 – 10 weeks old younger mice. In M3KO males, the PVRs increased by age; the PVRs at 1.5 years old elderly mice were 5.5 times greater than those at 10 – 12 weeks old younger mice. In females, in contrast, the PVRs of the M3KO mice were similar to those of WT during 5 – 10 weeks old younger mice, and did not increase with age. Significant gender difference was found in the compensation of the urinary dysfunction with increased PVR caused by the lacking of M3 mechanism（$p<0.01$）. Administration of the ATR and SS significantly increased the PVR in M3KO mice of both sexes, suggesting that the urinary dysfunction by the lacking of M3 mechanism was compensated by other muscarinic receptor pathway (instead of M3) and prostaglandins (PGs) pathways, respectively. In comparison of the compensatory role between ATR versus SS, administration of SS increased PVR significantly ($p<0.01$) than administration of ATR in M3KO female mice (Figure 2).

Interpretation of results

In male M3KO mice, the lack of M3 muscarinic acetylcholine receptor resulted in significant increase of PVR; on the other hand, in female M3KO mice which demonstrated minimum PVR similar to wild female mice, PGs mediated pathway and other muscarinic receptors mediated pathways (such as M2 instead of M3) played main compensatory roles for the M3 mediated urinary dysfunction. In such compensatory mechanism in M3KO mice, the PG mediated pathway could play greater role than the other muscarinic receptors mediated pathways.

Concluding message

Transrectal ultrasonic imaging analysis of the bladder is a useful modality to monitor the urinary function in conscious mice. In M3KO male mice, abnormally increased residual urine existed at infancy and increased with aging. The PG receptor mediated pathway was mainly involved in the mechanisms to compensate for the loss of M3 mediated urinary function. Such compensatory mechanisms were found to be superior in female mice than in male mice.

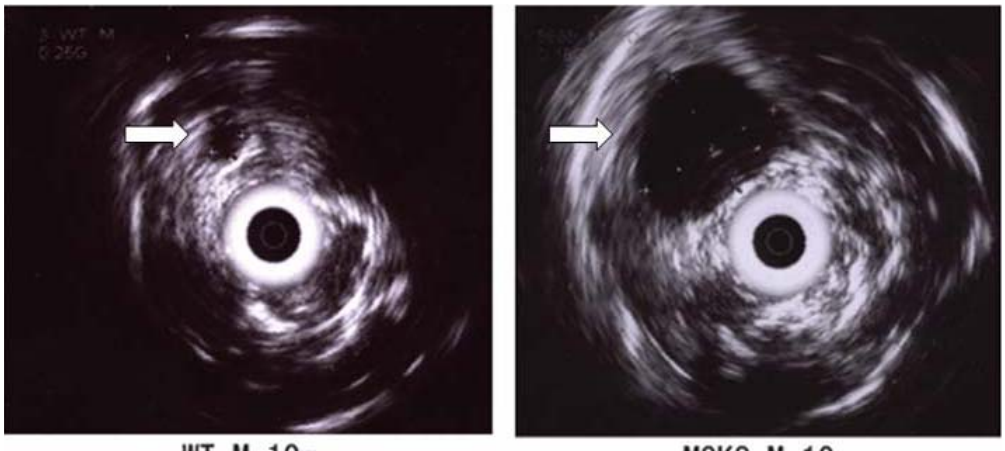

Figure 1. Postvoid residual urine (PVR) measured by miniature TRUS at 10 weeks old. Left image: wild type male. Right image: M3KO male. Arrows indicate the bladder with PVR.

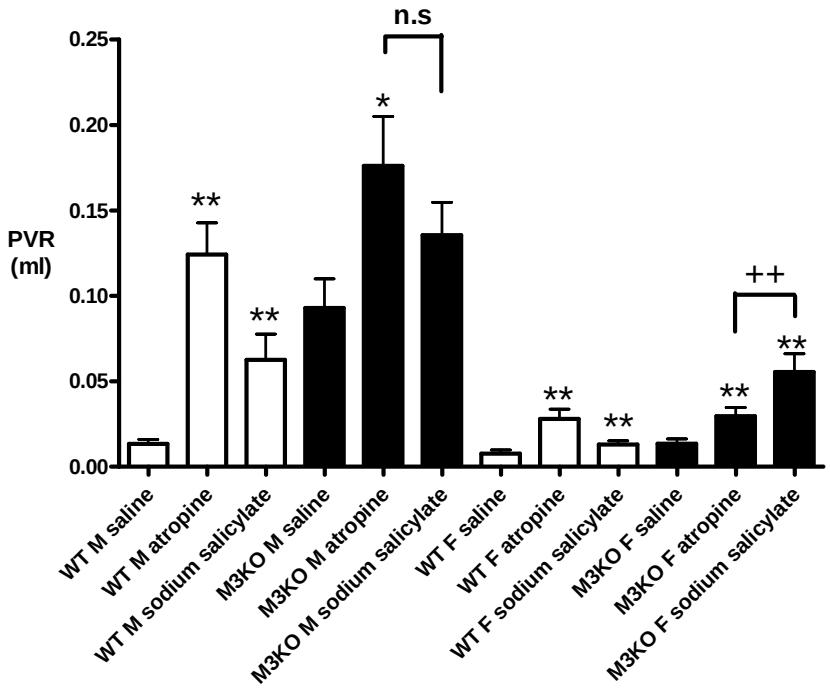

Figure 2. Postvoid residual urine (PVR) after subcutaneous administrations of saline, atropine, and sodium salycylate, in comparison between wild type (WT) and M3KO mice in both sexes (M, male; F, female). *; $p<0.05$ and **; $p<0.01$ comparison with saline, ++; $p<0.01$

References

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ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Institutional Animal Care and Use Committee policies of Tokyo University