67

Furuno T[1], Kakizaki H[2], Tanaka H[1], Mitsui T[1], Kitta T[1], Matsui M[3], Nonomura K[1]
1. Department of Urology, Hokkaido University Graduate School of Medicine, 2. Department of Urology, Asahikawa Medical College, 3. Division of Neuronal Network, Institute of Medical Science, University of Tokyo

ENHANCED INHIBITORY EFFECTS OF BETA-ADRENOCEPTOR AGONIST ON CHOLINERGIC MICTURITION CONTRACTIONS IN MUSCARINIC M2 RECEPTOR KNOCKOUT MICE.

Hypothesis / aims of study

Muscarinic M_2 receptor is coupling with G_i to inhibit adenylyl cyclase and decreases cAMP levels, and beta-adrenoceptor is coupling with G_s to activate adenylyl cyclase and increases intracellular cAMP levels. Increase of cAMP mediates relaxation of the bladder smooth muscle. Thus, M_2 receptor and beta-adrenoceptor are counteracting each other via adenylyl cyclase activity. A previous in vitro study showed an increased relaxant action of isoproterenol (beta-adrenoceptor agonist) on muscarinic agonist-induced contraction of the bladder smooth muscle from M_2 receptor knockout mice [1]. This result suggests that cholinergic contractile mechanism in the detrusor smooth muscle involves M_2 receptor-mediated inhibition of the relaxant effects of beta-adrenoceptor activation. The aim of this study is to investigate the relationship between M_2 receptor and beta-adrenoceptor in bladder functions in vivo.

Study design, materials and methods

Eight to 10-week male muscarinic M_2 receptor knockout mice (M_2KO) and wild type C57BL/6 (WT) were used in this study. A mutant mouse line was backcrossed to C57BL/6 at least 10 generations and established as a congenic line. Conscious filling cystometry was performed via cystostomy catheter surgically inserted on the day with isoflurene inhalation. After 1 to 2 hour wait, saline of room temperature (1.5ml/h) was infused and bladder pressure was measured. To evaluate the isoproterenol effects, after baseline measurement, vehicle (saline), 10, 100 and 1000 µg isoproterenol was cumulatively injected subcutaneously (WT n=10, M_2KO n=11). To evaluate the contribution of cholinergic and purinergic component, the effects of atropine (1 mg/kg s.c.) and additive alpha, beta-methylene-ATP (abMeATP, 3 mg/kg i.p) on micturition contraction were investigated in 5 WT mice. The effects of isoproterenol on purinergic and cholinergic component of contraction were evaluated. The differences between WT and M_2KO were analyzed.

Results

There was no difference between WT and M_2KO on baseline cystometry. Micturition pressure after administration of isoproterenol 100 and 1,000 µg/kg were significantly lower in M_2KO than in WT (100 µg/kg: WT 26.3+-1.6 cmH_2O vs. M_2KO 21.8+-1.2 cmH_2O, $p<0.05$; 1,000 µg/kg: WT 24.7+-1.5 cmH_2O vs. M_2KO 18.0+-1.0 cmH_2O, $p<0.01$; see figure 1). After administration of maximum dose isoproterenol, residual volume were higher (1000 µg/kg: WT 10.4+-1.6 µl vs. M_2KO 15.1+-1.4 µl, $p<0.05$) and voiding efficacy were lower (1000 µg/kg: WT 86.9+-2.2 % vs. M_2KO 79.5+-1.7 %, $p<0.05$) in M_2KO than in WT. Other cystometric parameters had no significant difference between WT and M_2KO.
Biphasic and rhythmic micturition contractions were observed during conscious filling cystometry and atropine (1 mg/kg s.c.) gradually inhibited the second phase contractions (before atropine: 30.8+-1.3 cmH_2O) in each mouse to undetectable level, whereas there was no significant effect on the initial phase contraction (before atropine: 19.0+-0.7 cmH_2O; after atropine: 16.8+-0.8 cmH_2O; see figure 2). Additive administration of abMeATP completely abolished the rhythmic contractions after transient bladder pressure increase in 4 of 5 WT mice (figure 2). Thus the initial contraction was ATP sensitive and the second phase contraction was atropine sensitive. M_2KO as well as wild type mice exhibited biphasic micturition contractions during cystometry.
Isoproterenol (1,000 µg/kg s.c.) had no significant effect on the amplitude of the initial phase contraction, but clearly inhibited the second phase contraction in both WT and M_2KO (figure 3). However, this inhibitory effect of isoproterenol on the second phase contraction was more prominent in M_2KO than in WT (isoproterenol 1,000 µg/kg: WT 22.5+-1.4 cmH_2O vs. M_2KO 14.6+-1.5 cmH_2O, $p<0.01$; figure 3). These results revealed that inhibitory effects of isoproterenol on cholinergic micturition contractions were enhanced in the absence of muscarinic M_2 receptor.

Interpretation of results

Primal bladder function in muscarinic M_2 receptor knockout mice is identical to wild type, but the inhibitory effect of beta-agonist, isoproterenol on the second phase cholinergic detrusor contraction is more pronounced in muscarinic M_2 receptor knockout mice.

Concluding message

Although the specific functions of each muscarinic receptor subtype had been mainly investigated in vitro, the present study showed the enhanced inhibitory action of isoproterenol on cholinergic micturition contraction in muscarinic M_2 receptor knockout mice in vivo. This result suggests that cholinergic contractile mechanism of micturition involves M_2 receptor-mediated inhibition of the relaxant effects caused by beta-adrenoceptor activation. Suitable balance between M_2 receptor and beta-adrenoceptor via the change of adenylyl cyclase activity may play a essential role for normal bladder functions.

References

(1) JPET 305: 106-113, 2003

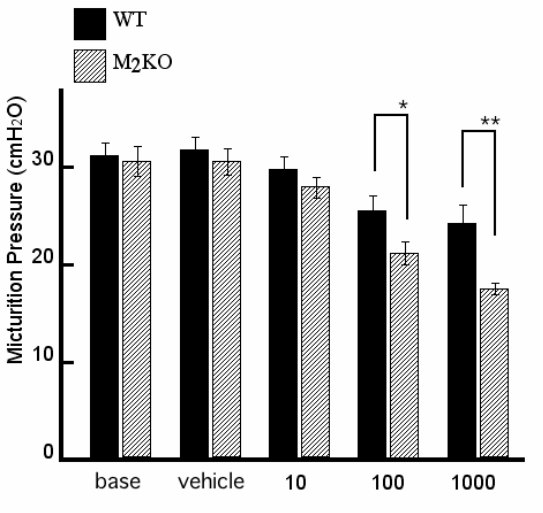

Figure 1: Inhibitory effects of isoproterenol on micturition pressure was more conspicuous in M_2KO.
*p<0.05 and **p<0.01

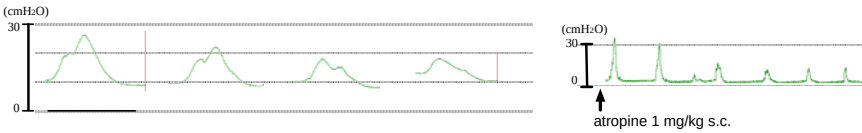

Figure 2: Effects of atropine and abMeATP on micturition in wild type. Atropine inhibited second phase contraction (left) and additive abMeATP suppressed rhythmic micturition after transient increase of bladder pressure (right).

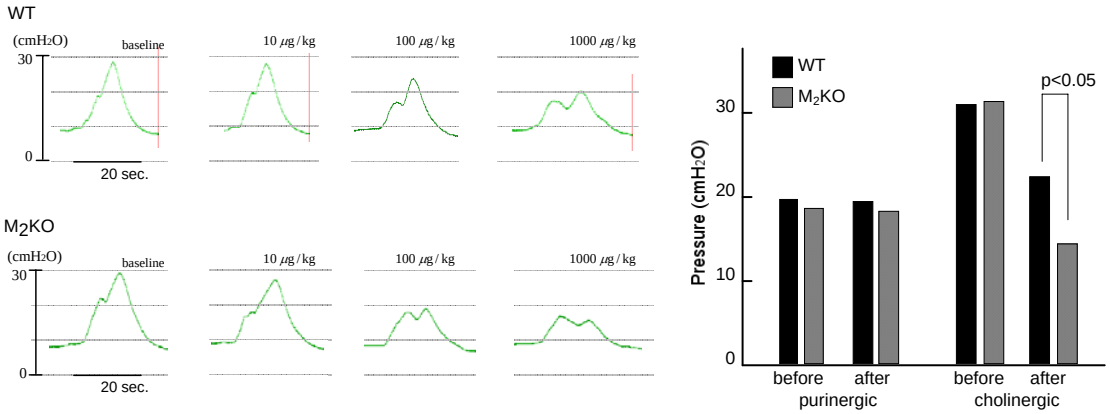

Figure 3: Cumulative dose of isoproterenol inhibited second phase cholinergic contraction and this effect is more pronounced in M_2KO.

FUNDING: **Pfizer, Pharmacia, The New Energy and Industrial Technology Development Organization (NEDO) of Japan, The Ministry of Education, Culture, Sports, Science and Technology (MEXT)**
DISCLOSURES: **NONE**
ANIMAL SUBJECTS: **This study followed the guidelines for care and use of laboratory animals and was approved by Hokkaido University Institutional Animal Care and Use Committee**