59

Authors: M. Yoshida, H. Iwashita, M. Otani, Y. Kawano, K. Miyamae and S. Ueda
Institution: Department of Urology, Kumamoto University School of Medicine
Title: IN VIVO MUSCARINIC M3 RECEPTOR GENE TRANSFER INTO RAT BLDDER SMOOTH MUSCLE BY ELECTROPORATION

Aims of study

Bladder smooth muscle contraction is mainly mediated by muscarinic m3 receptor. It may be possible that m3 receptor gene transfer into bladder smooth muscles may increase detrusor contractility, and become one of useful treatment modalities for weak detrusor. Recently, several reports have suggested that in vivo gene transfer by electroporation is effective for introducing DNA into mouse skin, skeletal muscles and rat liver (1-3). Based on these findings, we have examined the efficacy of this method for in vivo gene transfer into bladder smooth muscle, and attempted to transfer muscarinic m3 receptor gene into bladder smooth muscles in rats.

Methods

In adult male rats, small abdominal midline incision was performed, and bladder was separated under pentobarbital anesthesia. For the transfers of the marker gene (green fluorescent protein: GFP) and rat muscarinic M3 receptor gene, 50 μg GFP plasmid DNA and M3 receptor plasmid DNA, were injected into anterior and posterior subserosal space of the bladder by using a 27G needle. Then, the whole bladder was pulsed twice from a T820 square wave electroporator using a dish shaped electrode. Four square-wave pulses were delivered at a frequency 1 Hz. with pulse length of 50 msec and 30 V. In the control rats, each gene plasmid was injected without pulsing. Three days after each plasmid DNA injection, rats were sacrificed and bladder was isolated. GFP expression in the bladder specimen was visualized with a confocal microscope with excitation at 488 nm. The contractile responses induced by KCl (80mM), carbachol (0.01 10μM and electrical field stimulation (EFS: supramaximum voltage, duration 0.3 msec, 5 60 Hz, 3 sec train) were evaluated in smooth muscle strips isolated from bladder of the control rats and rats transferred m3 receptor plasmid DNA. Furthermore, one week after the gene transfer, under urethan anesthesia, cystometric evaluation was performed in both groups.

Results

Bladder specimens received GFP plasmid DNA injection with electroporation, showed bright and numerous GFP-expressing smooth muscles in deep bladder layer. In contrast, the control rat bladder, which received only GFP plasmid DNA injection, expressed only a few punctuate dots of green signals. In the functional experiments, KCl-induced contractile responses of smooth muscle strips were not significantly different between bladder injected m3 receptor plasmid DNA with electroporation and bladder without electroporation. However, the contractile responses induced by carbachol and EFS were significantly greater in bladder injected m3 receptor plasmid DNA with electroporation, as compared to bladder without electropolation (Table). In the findings of cystometrograms, micturition pressure and volume were significantly increased in rats transferred m3 receptor plasmid DNA, as compared to the control rats (Table).

Table: Effects of muscarinic m3 receptor gene transfer on contractile responses in rat detsusor smooth muscles and cystometric findings.

	Rats transferred m3 receptor	Control rats
In vitro functional experiment (contractile responses; g/mm^2)		
KCl (80 mM)	4.56±0.53	4.84±0.60
Carbachol (100 μM)	7.43±1.23*	5.19±0.73
EFS (60 Hz)	5.06±0.83*	3.74±0.53
Cystometric findings		
Micturition pressure (cmH_2O)	59.6±6.3*	45.6±6.5
Micturition volume (ml)	0.38±0.05*	0.28±0.03

Values are means±S.E.M..

* Significantly different from the comparable value of control rats ($p< 0.05$).

Conclusions

These data demonstrated that electroporation is useful method for in vivo gene transfer into bladder smooth muscles, and that m3 receptor gene transfer into rat bladder using this method could enhance muscarinic receptor-mediated contractile responses of bladder smooth muscles.

References

1. Gene Therapy 6: 508-514, 1999.
2. Proc. Natl. Acad. Sci. USA 97: 354-359, 2000.
3. FEBS 425:436-440, 1998.

Source of funding: None