501

Iwashita H, Yoshida M, Otani M, Kawano Y, Miyamae K, Murakami S, Masunaga K, Ueda S
Urology, Kumamoto University School of Medicine

IN VIVO NEURORAL NOS EXPRESSION VECTOR TRANSFER INTO RAT BLADDER BY ELECTROPOLATION

Aims of Study

Recently, we have attempted in vivo gene transfer by electroporation. In the previous study, we demonstrated that muscarinic m3 receptor gene transfer into rat bladder using this technique could enhance muscarinic receptor-mediated contractile responses of bladder smooth muscles (1). Nitric oxide (NO) is one of important neurotransmitters in lower urinary tract function. However the role of NO in bladder function was not clearly elucidated. Recently we have suggested that NO inhibits of acetylcholine release from cholinergic nerve endings in rabbit bladder smooth muscles, and contribute to the urinary filling mechanism (2). In the present study, we have made neuronal NO synthase (NOS) expression vector, and performed to transfer this vector into rat bladder in vivo.

Methods

In 8 week adult male rats, small abdominal midline incision was performed, and bladder was separated under pentobarbital anesthesia. For the transfers of the marker genes (green fluorescent protein: GFP and luciferase) and rat neuronal NOS gene, 50 μg each plasmid DNA was 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. Eight square-wave pulses were delivered at a frequency 1 Hz. with pulse length of 50 msec and 50 V. Rats were divided into four groups 1) vector injection with electroporation, 2) electroporation only, 3) vector injection only and 4) sham operated normal control. 72 hours 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. Luciferase activity was measured by Promega assay kit luciferase, (plasmid pGL3), and luminometer (Luminocounter 700, NITI-ON, Japan). The contractile responses induced by KCl (80 mM), 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 each group rat. Furthermore, the microdialysis prove was inserted into the bladder muscle strips, and Ringer solution was perfused into the probe at a rate of 2μl/min. The perfused solution during EFS was collected every 10 min, and the 20μl of the solution was injected into HPLC. The amount of NO_2^-/NO_3^- (NOx) was measured by NOx analyzer based on Griess method.

Results

Bladder specimens received GFP plasmid DNA injection with electroporation showed bright and numerous GFP-expressing smooth muscles in deep bladder layers. Luciferase activity was detected only in bladder from rat injected vector with electroporation. In the functional experiments, KCl- and EFS-induced contractile responses of smooth muscle strips were not significantly different among groups. In the group of NOS vector injection with electroporation, the amount of basal NOx release from bladder stirp was higher, as compared to the other groups. EFS caused increases in NOx releases from bladder strips in all groups. The increase rate was higher in the group of NOS vector injection with electroporation, than in other groups.

Conclusions

The present data suggest that electroporation is a useful technique for in vivo gene transfer into bladder smooth muscles, and that neuronal NOS gene transfer using this technique may cause functional expression of NOS gene in rat bladder.

References

1) Neurourol. Urodynam. 20: 465. 2001
2) Eur. J. Pharmacol. 428: 59. 2001