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IN VIVO NNOS GENE TRANSFER INTO THE RAT BLADDER USING ELECTROPORATION

Hypothesis / aims of study

Recently, we have attempted in vivo gene transfer by electroporation. In the previous study, we had made neuronal NO synthase (nNOS) expression vector, and performed to transfer this vector into rat bladder by electroporation, and confirmed nNOS gene expression. Our recent study showed that NO prejunctionally inhibited acetylcholine release from cholinegic nerve endings in rabbit bladder smooth muscles (1). The data suggest that NO may be an important modulator for afferent nerve function, and that have the regulatory effect of other neurotransmitters releases. In the present study, we performed to transfer nNOS expression vector into rat bladder, and we evaluated bladder function by cystometric study, and measured NOx release from the rat bladder.

Study design, materials and methods

Adult female SD rats were used in this study. For the transfers of neuronal NOS gene, 100mg plasmid DNA was injected into subserosal space of the bladder, and electroporation was performed as reported previously (2). To examine the expression of nNOS in the rat bladder, immunohistochemical analysis using anti-nNOS protein antibody were performed. 72 hours later, they were anesthetized with 0.9mg/kg urethane, and the lower abdominal cavity was opened with a mid-line incision, bilateral ureters were ligated and dissected, and intravesical pressure was recorded. To measure intravesical pressure in the urinary bladder, a 20G cannula was transurethrally inserted into the bladder, and was connected to a pressure transducer. Physiological saline was continuously perfused at a rate of 3 ml/hour. To measure NOx release in filling phase, we used in vivo microdialysis technique. The microdialysis probe was inserted into the bladder wall and the inlet cannula of the probe was connected to a microinfusion syringe pump. Ringer solution was continuously perfused at a rate of 2 microliter/min. Bladder volume was adjusted 1.0 ml by the infused volume of physiological saline, and dialysate solution was collected into microtube. The amount of NOx in the dialysate fraction was measured by HPLC based on Griess method.

Results

In nNOS immunohistochemical staining assay, bladder smooth muscles were significantly stained by anti-nNOS protein antibody in the nNOS transferred rat, as compared to the control group. In the cystometric findings, interval of micturition tended to increase, however, micturition pressure was not significantly different from control rats. The amount of NOx release from bladder of nNOS gene transferred rats was significantly higher than that from the control rat

Interpretation of results

The data of immunohistochmeical study and NOx release experiment from the rat bladder demonstrate that the transferred nNOS genes are functionally expressing. Furthermore, the cystometric findings suggest that the increased NO releases may increase in bladder capacity without any effects on the micturition pressure. As reported previously, increased NO release may prejunctionally inhibit acetylcholine release from cholinegic nerve endings in the filling phase.

Concluding message

In vivo nNOS gene transfer into rat bladder by electroporation causes functional expression of NOS gene in the bladder. The increased NOx release in the bladder may inhibit the detrusor overactivity in the filling phase.

References

1. Eur. J. Pharmacol. 428: 59-67, 2001
2. BJU int. May 2004 (in press)