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NEURONAL NO SYNTHASE GENE TRANSFER INTO RAT PROSTATE WITH IN VIVO ELECTROPORATION

Aims of Study

Recently, we have attempted in vivo gene transfer by electroporation (EP) procedure. In the previous study, we demonstrated that muscarinic M_3 receptor gene transfer into rat bladder using this method could enhance muscarinic receptor-mediated contractile response of bladder smooth muscles. It has been reported that nitric oxide (NO) mainly contributes to prostate or urethral smooth muscles relaxation. It is supposed that NO synthase (NOS) gene transfer into prostate would decrease in prostatic urethral tone. In the present study, we have made neuronal NOS (nNOS) expression vector, and attempted to transfer this vector into rat prostate using in vivo EP procedure.

Methods

Male Sprague-Dawley rats (200-250 g) were divided into four groups; sham group (sham), EP only group (EP only), nNOS gene transfer only group (nNOS only), nNOS gene transfer with in vivo EP group (nNOS+EP). Animals were anesthetized with the intraperitoneal injection of pentobarbital sodium (30 mg/kg) using a 26-gauge needle. A lower abdominal midline incision was made, and the prostate was identified. Fifty μg of reporter and nNOS gene expression plasmids in 50 μl of K-PBS (NaCl 30.8 mM, KCl 120.7 mM, Na_2HPO_4 8.1 mM, KH_2PO_4 1.46 mM and $MgCl_2$ 10 mM in distilled water) was injected into rat prostate. Immediately after the injection of these plasmids, the electrodes with 3 mm space surrounded the plasmid injection points. Square electric pulses were applied with an electroporator. After the intraperitoneal injection of gentamicin sulfate (0.5 mg/kg), the muscle and skin were sutured with 4-0 absorption threads. Two days after gene transfer, rats were sacrificed, and we performed reporter gene expression analyses and an immunohistochemical analysis. Furthermore NO_2^- /NO_3^- (NOx) release from isolated prostate strip of each group was evaluated using high-performance liquid chromatography coupled with a microdialysis procedure. The amount of NOx was measured by NOx analyzer based on Griess method. Neuronal NOS gene transfer experiments were performed under the optimal pulse conditions obtained by the luciferase assay.

Results

The results of luciferase assay showed that optimal conditions of electroporation were 8 pulses, 50 voltages, 10 milliseconds /pulses and 1 Hz for gene transfer into rat prostate. Under these conditions, luciferase gene expression was enhanced approximately 300-fold, as compared to nNOS only group. Immunohistochemical analysis of prostate showed that the marked nNOS staining in nNOS+EP group, as compared to other groups. NOx release from the isolated prostate strip was significantly higher in nNOS+EP group than that in other groups (Table. 1).

Table 1. NOx release from rat prostate strips in four groups

	Sham	EP only	nNOS only	nNOS + EP
NOx release (pmol/g)	51.20 ± 9.60	46.07 ± 9.16	40.61 ± 7.11	98.55 ± 9.33*

Each strip was stretched about 0.5 g resting tension. NOx release represents mean ± S.E. of ten experiments. * $P < 0.05$: significantly different from values of other groups.

Conclusions

These findings suggest that in vivo EP procedure is useful method for gene transfer into prostate, and that nNOS gene transfer using this method causes functional expression of NOS gene in rat prostate.