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ELECTROPORATION-MEDIATED MUSCARINIC M3 RECEPTOR GENE THERAPY FOR DENERVATION BLADDER IN THE RAT MODEL

Hypothesis / aims of study

Muscarinic M_3 (M_3) receptor has been recognized as a major muscarinic receptor for smooth muscle contractions of the urinary bladder. It has also been reported that a decrease in M_3 receptor of bladder outlet obstruction (BOO) and denervation bladder causes detrusor underactivity. Recently, we have attempted in vivo gene transfer by electroporation (EP) procedure. In this study, to investigate the possibility of muscarinic M_3 (M_3) receptor gene therapy, we attempted to transfer M_3 receptor gene into partially denavated bladder using electroporation (EP) procedure.

Study design, materials and methods

Eight-week female rats (200-250 g) were anesthetized with the intraperitoneal injection of pentobarbital sodium (30 mg/kg) using a 26-gauge needle. A midline incision was made in the lower abdomen. The pelvic plexus was exposed. For partially denavated bladder, the right major pelvic ganglion with its nerve filaments close to the bladder was resected. Twelve weeks after the denervation, M_3 receptor gene was transferred into partially denavated bladder using EP procedure. Ten days after gene transfer, we performed cystometric study and the functional studies using bladder strips isolated from both M_3 receptor gene transfer, denervated and control rats. Moreover, M_3 receptor mRNA levels also were determined by quantitative real-time RT-PCR.

Results

Five weeks after denervation, M_3 receptor mRNA levels in rats with partially denervated bladder were lower than that of control rats. Partial denervation caused a decrease in micturition pressure in cystometrogram, and decreases in carbachol- and EFS-induced contractions in the functional study. In partially denervated bladder transferred M_3 receptor gene, M_3 receptor mRNA levels were significantly higher than those of other groups (Table 1). In rats transferred M_3 receptor gene, carbachol- and EFS-induced maximum contractile responses of bladder smooth muscle strips were significantly increased, as compared to those of other groups (Table 2). Cystometric findings in rats with M_3 receptor gene transfer showed an increase in micturition pressure.

Interpretation of results

In this study, partial denervation induced decreases in M_3 receptor mRNA levels, the contractile responses and extrinsic muscarinic receptor-mediated stimulation. Overexpression of muscarinic M_3 receptor gene in partially denervated bladder restored the contractility.

Concluding message

In vivo EP is a useful procedure for gene transfer into rat bladder. M_3 receptor gene transfer using this procedure may provide a new treatment modality for detrusor underactivity due to decreased number or function in M_3 receptor.

Table 1 M_3 receptor mRNA levels by quantitative real-time RT-PCR

Group	M_3 receptor mRNA levels (M_3R, mean/β-Act, mean)
Sham	0.98±0.05
DEN (12 week)	0.68±0.08
M_3R + EP	5.17±2.25∗

Sham, sham operation; DEN (12 week), 12 week after the denervation; M_3R + EP, muscarinic M_3 (M_3) receptor gene transfer using in vivo EP; β-Act, β-Actin. Each value represents the mean ± S.E. of ten experiments. ∗ indicates statistically significant difference from other groups ($P < 0.05$).

Table 2 Maximum contractile responses induced by carbachol and electric field stimulation (EFS) in rat bladder smooth muscles of five groups

Group	Carbachol (% 80 mM KCl contraction)	EFS (% 80 mM KCl contraction)
Sham	138.95±6.78	150.33±18.57
DEN (12 week)	112.16±11.16	84.24±10.85
M_3R + EP	171.73±7.44	147.49±6.35

Sham, sham operation; DEN (12 week), 12 week after the denervation; M_3R + EP, muscarinic M_3 (M_3) receptor gene transfer using in vivo EP. Each value represents the mean ± S.E. of ten experiments.