

BASIC FIBROBLAST GROWTH FACTOR (bFGF) COULD BE A KEY REGULATOR FOR MYOGENIC BLADDER OVERACTIVITY THROUGH GAP JUNCTION GENERATION IN THE SMOOTH MUSCLE.

Hypothesis / aims of study

Detrusor overactivity is a highly prevalent clinical condition in bladder outlet obstruction (BOO) and result from neurogenic changes in detrusor innervation or myogenic changes in detrusor excitability [1]. Increased coupling of bladder smooth muscle cells (BSMC) through gap junctions has been hypothesized as a mechanism for myogenic bladder overactivity in BOO [2], although little is known about the regulatory system underlying such changes. Meanwhile, our previous study showed that bFGF up-regulation in BOO induced alteration in collagen production and BSMC proliferation [3]. Hence, we investigated regulatory role of bFGF for detrusor overactivity and involvement of connexin 43, a gap junction protein.

Study design, materials and methods

1) *Rat BOO model*: Partial BOO was created by urethral constriction (n=6). The expression of bFGF, connexin 43, and calponin, a smooth muscle cell marker, was determined by immunostaining. Contractile response of bladder strips to carbachol stimulation was assessed.

2) *In vitro effect of bFGF*: Rat BSMC were treated with bFGF (0 and 10 ng/ml) in the presence or absence of anti-bFGF antibody or inhibitor of ERK1/2, its putative downstream signal (n=3). Connexin 43 expression was assessed by immunoblotting and staining.

3) *In vivo effect of bFGF*: Acidic gelatin hydrogel was employed as a release carrier of bFGF (sham, 0, and 10 µg/site), and was fixed over rat bladder for 14 days (n=6). The expression of connexin 43 and calponin was assessed by immunostaining. Gap junction formation was assessed by electron microscopy. Contractile response of bladder strips to carbachol was assessed in the presence or absence of a gap junction inhibitor, 18β-glycyrrhetic acid (β-GA). Filling cystometry was also performed.

Results

1) bFGF and connexin 43 was up-regulated in the obstructed rat bladders compared to sham control bladders. Most connexin 43-positive cells were also positive for calponin. Obstructed bladders showed hypersensitivity to calbachol, as demonstrated by a leftward shift in the dose-response curve and higher pEC50 value of carbachol concentration.

2) In vitro, bFGF induced connexin 43 up-regulation in BSMC, which was reversed by anti-bFGF Ab or ERK1/2 inhibitor.

3) In vivo, bFGF induced connexin 43 up-regulation and gap junction formation in the smooth muscle layer (Fig. A). bFGF treatment also induced hypersensitivity to calbachol which was blocked by inhibition of gap junction with β-GA (Fig. B), but β-GA did not affect maximum contractile force. Cystometric analyses of bladders treated with bFGF demonstrated significantly more frequent micturition and less bladder capacity, which were two typical features of detrusor overactivity.

Interpretation of results

Rat BOO model study indicates that bFGF up-regulation, connexin 43 up-regulation in BSMC, and hypersensitivity to cholinergic agents are simultaneous phenomena in detrusor overactivity. bFGF treatment in vitro and in vivo shows the etiological evidence that bFGF induce connexin 43 expression in BSMC associated with hypersensitivity to cholinergic agents. Those findings and detrusor overactivity in bladders treated with bFGF suggest that bFGF could be a pivotal signal for induction of myogenic detrusor overactivity in BOO through altered gap junction protein in BSMC.

Concluding message

This study provides molecular clues for a new idea of pharmacological gap junction inhibition to overactive bladder, since blockade of gap junction activity altered hypersensitivity in bladders treated with bFGF without changes in maximum contractile force.

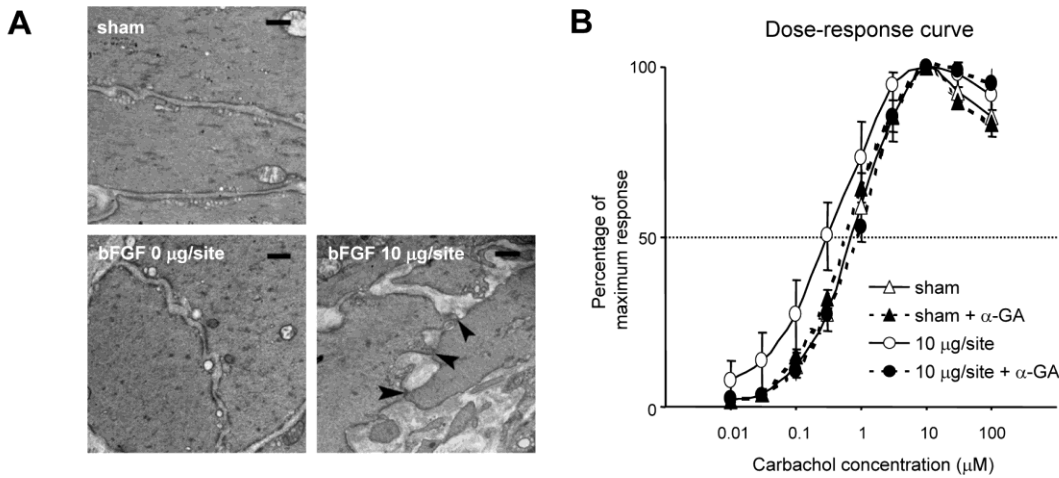


Fig. A: Ultrastructural studies for bladders. Bladders treated with bFGF (10 μ g/site, lower right) represent gap junction formation (arrows) in the smooth muscle layer, but not control bladders (sham or bFGF 0 μ g/site).

Fig. B: Muscle strip test for bladders treated with bFGF. The contractile force of bladder strips was examined under carbachol stimulation. Bladders treated with bFGF demonstrated left-shift in the dose-response curve, an evidence of hypersensitivity. Blockade of gap junction activity with α -GA reversed this left-shift.

References

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Name of ethics committee	Kyoto University Animal Experiment Committee