156

Mori K[1], Noguchi M[1], Matsuo M[1], Nomata K[1], Kanetake H[1]
1. Department of Urology, Nagasaki university graduated school of biomedical sciences

INTERNALIZATION OF GAP JUNCTIONAL PROTEIN, CONNEXIN 43, MODULATED BY ERK 1/2 ACTIVATION IN RATS DETRUSOR MUSCLE WITH PARTIAL BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study

Gap junctions are formed by connexons on the cell membranes of contiguous cells, each of which is a hexamer of connexin (Cx) proteins. Cell-to-Cell communication through junctional channels might play a crucial role in the control of cell growth, development, and differentiation in multicellular organisms [1]. Gap junctional intercellular channels allow direct movement between neighboring cells of ions, molecules, and neurotransmitters less than 1Kda [2]. Muscle cell actions are thought to be synchronized by promotion of gap junctional intercellular communication (GJIC). Furthermore, it is known that the activation of extracellular signalregulated kinase (ERK) 1 and ERK2 via epidermal growth factor receptor (EGFR) regulate phosphorylation and localization of gap junctional Cx-43 protein [3]. Signal transmission in detrusor muscle is unknown. We hypothesis that gap junctions in deturusor muscle may also play an important role in voiding function, and the alteration of gap junction in detrusor muscle was generated by ERK1 and ERK2.
Partial bladder outlet obstruction (P-BOO) secondary to benign prostate hypertrophy (BPH) is a common medical problem. Cell proliferation, hyperplasia and tones of detrusor muscle are modulated by P-BOO, BPH. In this study, we investigated the alterations of gap junctional protein, connexin-43 (Cx-43), and ERK 1/2 activation on the detrusor muscle of rat bladders with P-BOO.

Study design, materials and methods

Twelve week-old female Wistar rats divided into a P-BOO group (n=18) and a sham operated control group (n=15) were sacrificed 2, 4 and 8 weeks after surgery. For inducing partial bladder outlet obstruction, the rats were anesthetized by intraperitoneal injection of urethane 1g/Kg. The urethra was intubated with a 2.9F polyethylene tube. A midline abdominal incision was performed and the retropuvic space developed. The bladder was exposed, and a double 4-0 silk ligature was placed loosely around the proximal urethra producing a standardized degree of obstruction, and the tube was removed. The incisions were closed with surgical sutures. Sham-operated rats were underwent identical surgical procedures without ligation. After cystometric investigation, the entire bladder was removed and weighted. The expression of Cx-43 protein in the detrusor muscle was examined by immmunohistochemistry and Western blot analysis. Freeze-fracture analysis was performed to investigate whether morphological alteration of gap junction was detected. Furthermore, ERK 1/2 activation in the detrusor muscle was examined by Western blot analysis.

Results

Bladder weight and capacity were significantly greater in the P-BOO rats than the control rats ($P<0.01$). P-BOO rats at 8 weeks after surgery, no detrusor contraction was observed and voiding had the characteristics of overflow. Cx-43 plaques were expressed on the cell membranes of detrusor muscle cells in control rats and P-BOO rats at 2, 4 weeks. However, in only P-BOO rats at 8 weeks with no detrusor contraction, Cx-43 plaques were expressed in the cytoplasm and nuclei of detrusor muscle cells in immunohistochemistry. Freeze-fracture analysis could not also detected gap junction in detrusor muscle cells in P-BOO rats at 8 weeks. In control rats and P-BOO rats, P_0–Cx43 (unphosphorylated form), P_1–Cx43 (the phosphorylated form) and P_2-Cx43 (more the highly phosphorylated form) were detected at 2, 4, and 8 weeks after surgery in western blot analysis. The expression levels of P_0–Cx43 and P_1–Cx43 in P-BOO rats are higher than those of control rats respectively. Furthermore, the expression levels of Cx-43 protein (P0, P1) in P-BOO rats were gradually increased from at 2 weeks to at 8 weeks. Phosphorylated ERK1/2 was significant increased in P-BOO rats. The expression levels of phosphorylated ERK1/2 in P-BOO rats were gradually increased as Cx-43 expression from at 2 weeks to at 8 weeks.

Interpretation of results

This study demonstrated that gap junction on detrusor muscle in P-BOO rats was disrupted as a result of internalization of Cx-43 proteins. Furthermore, phosphorylated Cx-43 protein by ERK 1/2 activation was involved the internalization of Cx-43. Because of the disruption of GJIC, normal signals contributed the voiding function might not be transported between detrusor muscle.

Concluding message

These data suggest that the alteration of gap junctions modulated by ERK1 and ERK2 might be one of causing voiding dysfunction.

References

[1] Trosko, J.E., Madhukar, B.V., and Chang, C.C. 1993. Endogenous and exogenous modulation of gap junctional intercellular communication: toxicological and pharmacological implications. Life Sci. **53:** 1-19.

[2] Kumar, N.M., and Gilula, N.B. 1996. The gap junction communication channel. Cell. **84:** 381-388

[3] Mograbi, B., Corcelle, E., Defamie, N., Samson, M., Nebout, M., Segretain, D., Fenichel, P., Pointis, G. 2003. Aberrant Connexin 43 endocytosis by the carcinogen lindane involves activation of the ERK/mitogen-activated protein kinase pathway. Carcinogenesis. **24**: 1415-1423.