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THE CHANGES OF THE GAP JUNCTIONAL PROTEIN, CONNEXIN-43, IN RAT DETRUSOR MUSCLE WITH VOIDING DYSFUNCTION MODEL

Hypothesis / aims of study

Gap junctional intercellular communication may play an important role in cell growth, development and differentiation. Gap junctional intercellular channels allow direct movement between neighboring cells of ions, molecules and neurotransmitters less than 1.2 kDa. It is reported that the gap junctions may play an important role in synchronous cardiac myocytes contraction [1]. Gap junctions with direct movement of neurotransmitters between neighboring cells may also play an important role in synchronous detrusor muscle contraction in voiding function. Gap junction is composed with connexin (Cx) protein, the expression of Cx-26, Cx-43 and Cx-43 has also been demonstrated in human detrusor muscle cells. In rat detrusor muscle, Cx-43 is considered predominant connexin. It is also reported that gap junctions are regulated by extracellular signal -regulated kinase (ERK). We investigated the alteration of gap junctional protein, connexin-43 (Cx-43) in associated with ERK activation in rat detrusor muscle with voiding dysfunction.

Study design, materials and methods

Twelve week-old female Wistar rat were used in this study. Rats were divided into 3 groups: Partial bladder outlet obstruction (BOO) group, diabetes induced by streptozotosin (DM) group and control group. To obtain a BOO, the urethra was intubated with a PE-50 catheter, and a 4-0 silk ligature was placed loosely around the proximal urethra producing a standardized degree of obstruction and catheter was removed. Diabetes was induced in rats by intraperitoneal injection of 60 mg/Kg streptozotosin. Streptozotosin is dissolved in a 0.1M, pH 4.0 citrate phosphate buffer. Five days after injection of streptozotosin, rats glucose level was identified more than 300 mg/dl. Eight weeks later, cystmetrical investigation was performed under urethane anesthesia. After the cystmetrical investigation, bladder was removed. To analyze the alteration of the Cx-43 expression and extracellular signal-regulated kinase (ERK1/2) in detrusor muscle, immunohistochemical staining and western blot analysis were performed.

Results

Bladder weight and voided volume were significantly greater in both BOO rat and DM rat than the control rat ($P<0.01$). By the cystmetrical investigation, DM rats kept the detrusor contraction. However, no detrusor contraction was observed in only BOO rat at 8 weeks. Cx-43 was expressed in a dot like on cell membranes of the detrusor muscle in the control and the DM rats in immunohistochemical staining. However, Cx-43 plaques were visible in both the cytoplasma and the nuclei, and Cx-43 was revealed internal localization from cell membrane in BOO rats. The expression levels of Cx-43 of detrusor muscle in BOO rats were higher than those of the control and the DM rats. And the phosphorylated forms of Cx-43 band was more expressed in the BOO rats. Furthermore, ERK1/2 activation of detrusor muscle in only BOO rats.

Table. The changes of rat detrusor muscle with voiding dysfunction model

	Control rats (n=6)	BOO rats (n=6)	DM rats (n-6)
Bladder weight (g)	0.18 ± 0.1	1.60 ± 0.5	0.48 ± 0.1
Voided volume (ml)	0.5 ± 0.1	13.1 ± 3.1	5.8 ± 0.7
Detrusor contraction	normal	No contraction	normal
Immunohistochemical staining of Cx-43 (staining site)	Normal expression (cell membranes)	Internalization of Cx-43 (cytoplasma or nuclei)	Normal expression (cell membranes)
Western blot analysis of Cx-43	Normal expression	Highly expression and phosphorylation	Normal expression
Western blot analysis of	Normal expression	Activation	Normal expression

Interpretation of results

It is considered that the detrusor muscle contraction is kept via the gap junction. Gap junction is disrupted because of the internalisation of Cx-43 in BOO rats. It was reported that the internalisation of Cx-43 was caused to phoshporylation of Cx-43 forms. ERK 1/2 which is a member of the mitogen-activated protein kinase (MAPK) is thought to mediate Cx-43 phosphorylation [2]. In Boo rats, mechanical stretch for the detrusor muscle activates the ERK1/2, the activation of ERK 1/2 causes to the phosphorylation of Cx-43. Finally, internalisation of Cx-43 is induced by the phosphorylation of Cx-43, normal signals between detrusor muscles may not be transported.

Concluding message

Cx-43 of the detrusor muscle in the BOO rats may be regulated by the activation of ERK 1/2, and changes in Cx-43 were considered to cause disruptions of the gap junctions. These data suggest that the disruptions of the gap junction in the detrusor muscle may be one of the causes of voiding dysfunction.

References

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