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INTERNALIZATION OF GAP JUNCTIONAL PROTEIN, CONNEXIN-43 IN RAT WITH BLADDER OUTLET OBSTRUCTION

Aims of Study

Gap junction intercellular channels allow direct movement between neighboring cells of ions and molecules less than 1.2kDa [1]. These 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 [2,3]. It is reported that cardiac myocytes are interconnected in the synchronous contraction by gap junctional intercellular communication (GJIC) [4]. Gap junctions are thought to synchronize muscle cell actions by promoting intercellular communications. It is suggested that gap junction also will play an important role in voiding function. Bladder outlet obstruction (BOO) secondary to benign prostate hypertrophy (BPH) is common medical problem. It is estimated that more than 80% of males 60 years old and older have varying degrees of outlet obstruction secondary to BPH. However, there is no investigation about the GJIC of detrusor muscle with BOO bladder. Therefore, we investigated the alterations of gap junctional protein, connexin-43 (Cx-43) protein, on detrusor muscle of rat bladder with BOO.

Methods

The 12-week-old female Wistar rats were divided into two groups: a BOO group (n=13), a sham-operated control group (n=9). For inducing partial bladder outlet obstruction, the rat was anesthetized by intraperitoneal injection of urethane at 1g/Kg. The urethra was intubated with a 2.9F polyethylene tube. A midline abdominal incision was performed and the retropubic 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 polyethylene tube was removed. The incisions were closed with surgical sutures. Sham-operated rats were underwent identical surgical procedures without ligation. The BOO group and sham-operated control groups were sacrificed 2, 4 and 8 weeks after surgery. For cystometrical investigation, the bladder was gently exposed and intubated suprapubically with a 2.9F polyethylene tube under urethane anesthesia. After cystometorical investigation, the entire bladder was removed, and examined the expression of Cx-43 protein in detrusor muscle by immunohistochemistry and Western blot analysis.

Results

The weight of bladder was significantly increased in BOO group compared with sham-operated group ($P < 0.05$). In BOO group, the bladder capacity of rats after 4, 8 weeks was significantly higher than that of 2 weeks ($P < 0.05$). The detrusor contraction was not recognized and the voiding was overflow in only BOO rats after 8 weeks. In immunohistochemistry, Cx-43 protein was expressed on cell membrane of each sham-operated rats and the BOO rats after 2, 4 weeks. However, Cx-43 protein was stained in the cytoplasm or nuclei, not cell membrane in BOO rats after 8 weeks. In western blot analysis, three forms of Cx-43 protein, (unphosphorylated form P_0, the phosphorylated form P_1, and more highly phosphorylated form P_2), were detected in each sham-operated control group. In BOO rats after 2 and 4 weeks, three forms of Cx-43 protein were detected. However, the expression levels of unphosphorylated Cx- 43 in BOO rats after 8 weeks was significantly higher than in BOO rats after 2, 4 weeks.

Conclusions

Functional gap junction plaques should be localized to the cell membrane, and the aberrant localization of Cx-43 protein, such as in the cytoplasma or nuclei would be not functional [5]. Our results indicate that phosphorylation of Cx-43 protein was altered in detrusor muscle for the long term of bladder outlet obstruction. These data suggest that Internalization of gap junctional protein, connexin-43 in rat detrusor muscle with BOO for 8 weeks may contribute the disruption of GJIC function. It is concluded that the loss of GJIC function in detrusor muscle may be related to one of the cause for voiding dysfunction with continuous BOO.

References

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