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GAP JUNCTION REGULATES DETRUSOR ACTIVITY IN BLADDER INFLAMMATION.

Hypothesis / aims of study

Bladder inflammation could contribute to non-neurogenic mechanisms causing storage symptoms, because a close relationship between hyperactivity and inflammation in the bladder was indicated [1]. Meanwhile, previous studies demonstrated that gap junction proteins in the bladders function as key regulators for non-neurogenic mechanisms of various hyperactive bladder conditions [2, 3]. However, gap junction function in bladder inflammation is still unclear. The purpose of this study was to elucidate the role of gap junctions, as non-neurogenic mechanisms of storage symptoms, in bladder inflammation.

Study design, materials and methods

1) *Micturition analysis for mice model of cyclophosphamide (CYP)-induced cystitis*: Mice were injected intraperitoneally with 150 mg/kg body weight of CYP diluted in saline. Treated mice were kept in a cage located over the recording equipment for voided urine. Voiding behavior was analyzed under 12 hours light and 12 hours dark conditions (CYP; n=3, the sham-operated control group (CTRL); n = 4).
2) *Gap junction protein expression and formation analysis*: The bladders were manually dissected to two layers; urothelium with suburothelium (U), and smooth muscle layer (S). Immunoblotting of each layers were performed for expression of connexion 43 (GJA1), one of the major gap junction protein in the bladder (n=3). Whole bladders harvested from other mice were examined with transmission electron microscopy (TEM) for analysis of gap junction formation (n=3).
3) *Measurement of isometric tension*: Isometric force in one thread of the bladder was evaluated in the organ bath. Increasing concentrations of Bay K8644, an L-type Ca2+ channel opener, were cumulatively added and then stimulation was blocked by carbenoxolone disodium salt (CBX) (CYP; n=6, the sham-operated control group (CTRL); n = 5).
4) *Inhibition of gap junction function in vivo micturition analysis*: Voiding behavior was analyzed for CYP-treated mice with gap junction inhibition of 30 mg/kg body weight of 18α-glycyrrhetinic acid (GA) 24 hours prior to CYP administration (CYP+GA; n=5, as control (CYP); n=6).

Results

1) CYP-treated mice demonstrated a significant decrease in bladder capacity and increase in urinary frequency when compared to the control group.
2) Immunoblotting data revealed that GJA1 expression was stronger in the urothelium with suburothelium than in the smooth muscle layer in the CYP-induced cystitis group. However, TEM study revealed that up-regulated gap junction formation were observed in the bladder smooth muscle layer of the CYP-treated mice (Fig. 1).
3) Spontaneous contraction and its enhancement by Bay K8644 was observed in the CYP-treated mice. Bay K8644-induced spontaneous contraction was blocked by CBX in the CYP-treated mice (Fig. 2).
4) The CYP+GA group demonstrated a significantly increased volume at 48 and 60 hours when compared to the control group (Fig. 3).

Interpretation of results

The mouse model displayed some features of storage symptoms including voiding behavior and increased spontaneous contraction of bladder smooth muscle strips concurrent with up-regulated gap junction formation in the bladder smooth muscle layer. Inhibition of gap junction function in the mouse model attenuated spontaneous contraction and improved storage symptoms, indicating that gap junction function is a key regulator of storage symptoms in bladder inflammation.

Concluding message

Gap junction in the bladder might be an alternative therapeutic target for storage symptoms in bladder inflammation.

Fig.1

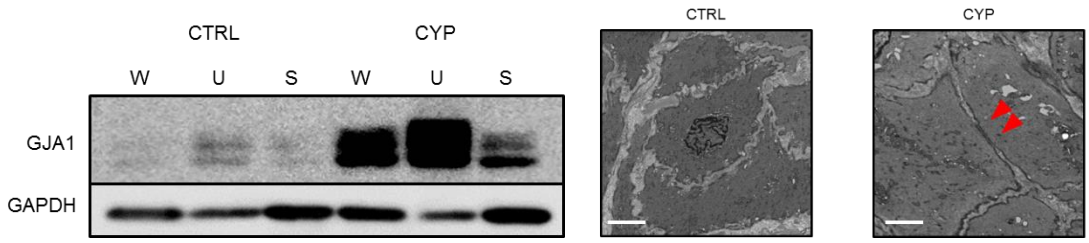


Fig.2

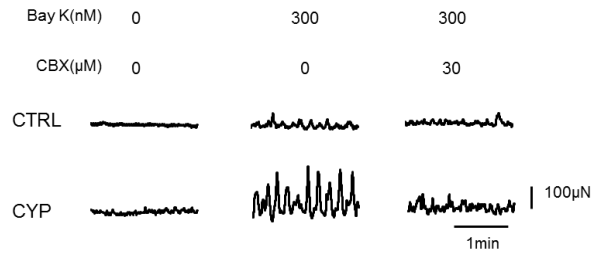
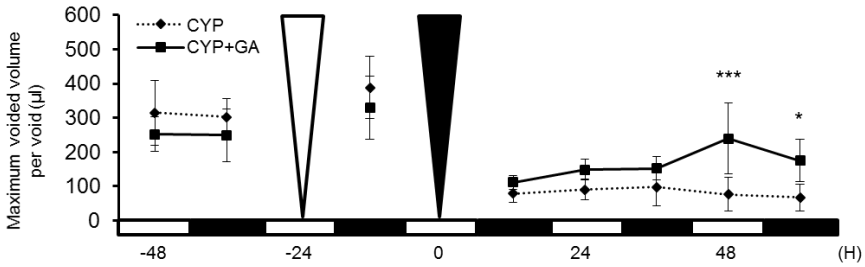


Fig.3



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Disclosures

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