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THE ROLE OF CHLORIDE CHANNELS ON CYCLOPHOSPHAMIDE-INDUCED OVERACTIVE BLADDER IN RATS

Hypothesis / aims of study
Overactive bladder (OAB) is mostly caused by detrusor uninhibited contraction. Previous study has demonstrated the important role of chloride channels on the regulation of urinary bladder smooth muscle tone. Whether chloride channel is involved in the pathogenesis of OAB has not been elucidated. We investigated the functional expression of CLC-3 and CLCA4 chloride channels on bladder tissue in rats with cyclophosphamide (CYP)-induced OAB.

Study design, materials and methods
A total of 96 adult male Wister rats (10–12 weeks) were divided into three groups (CYPc40, CYPc80 and control). CYP-induced OAB was provoked by four i.p. injections in 7 days (CYPc40: 40 mg/kg and CYPc80: 80 mg/kg). Control rat received saline injections. The experiments were performed on day 7. We conducted a strategy involving: (1)Continuous infusion cystometry (CMG) under anesthesia to record the basal pressure, threshold pressure, maximum bladder voiding pressure (MBVP) and intercontraction interval (ICI). (2) Urinary nerve growth factor (NGF) detection before CMG. (3)Western blot analysis and immunohistochemistry of CLC-3 and CLCA4 protein on rat bladder tissues. (4)Reverse transcription-polymerase chain reaction (RT-PCR) of the mRNA for CLC-3 and CLCA4 channels in normal and CYP-OAB bladder tissues. The CMG parameters, urine NGF level, molecular expressions of chloride channels are compared between rats in control, CYPc40 and CYPc80 groups.

Results
Repeated injection of low dose CYP (40 or 80 mg/kg) could successfully induce OAB like status in rats which was illustrated by CMG. In CYPc80 group, the bladder weight and urinary NGF increased significantly. In OAB rats (CYPc40 and CYPc80), western blotting showed the significantly increased protein expressions of CLC-3 and CLCA4 chloride channels on rat bladder tissue in OAB than in control groups in a dose dependent manner (Fig. 1). The Quantitative RT-PCR also detected significantly increased mRNA expression of CLC-3 and CLCA4 on bladder tissue in OAB than in control groups in a dose dependent manner (Fig. 2). Immunohistochemistry study revealed the CLC-3 and CLCA4 were located on both urothelium and smooth muscle layers in CYP-induced OAB bladder in rats. Moreover, the expression of CLC-3 and CLCA4 chloride channels both in protein and mRNA level on OAB rat bladder were strongly correlated with the NGF levels and CMG parameters (Fig. 3).

Interpretation of results
The current study showed that the CLC-3 and CLCA4 chloride channels were up-regulated in a dose dependent manner in both the mRNA and protein level in the OAB rat bladder tissues induced by CYP. The results indicate that these chloride channels are involved in the pathogenesis of OAB. In addition, the expressions (protein and mRNA) of CLC-3 and CLCA4 chloride channels were positively correlated with NGF levels, indicating the possible role of chloride channels on production of OAB biomarker. Moreover, the protein and mRNA expressions of CLC-3 and CLCA4 were negatively correlated with the CMG parameters, MBVP and ICI, demonstrating the effects of chloride channels on OAB symptoms.
Concluding message
Our results suggest that both the CLC-3 and CLCA4 chloride channels may play important roles in the pathogenesis of OAB which may provide a new therapeutic target in treating OAB.

Fig. 1. Western Blot Analysis of ClC-3 or CLCA4. Western blotting technique showed that both the CLC-3 and CLCA4 proteins on rat bladder tissues were significantly up-regulated in the CYP-treated groups in a dose dependent manner.

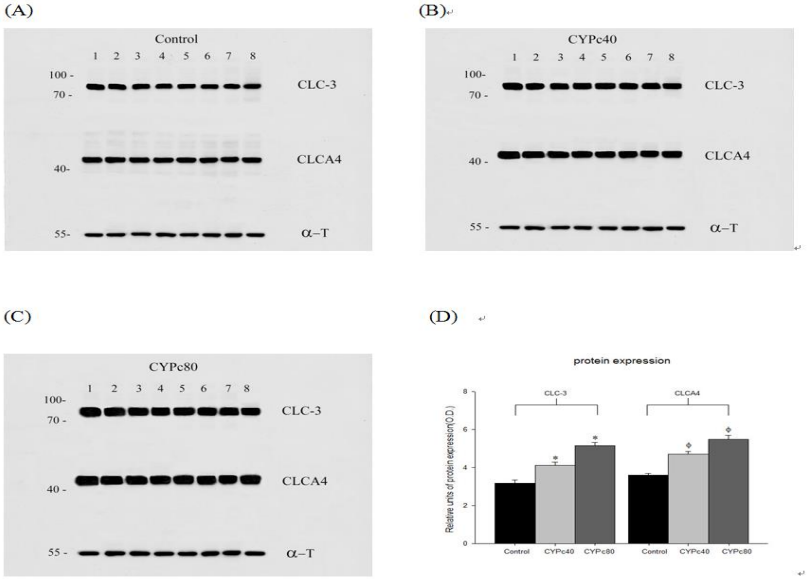


Fig.2. Quantitative RT-PCR Analysis of ClC-3 or CLCA4 mRNA. As compared to the control group, the mRNA expressions of ClC-3 and CLCA4 were significantly increased in the CYP-treated group in a dose dependent manner.

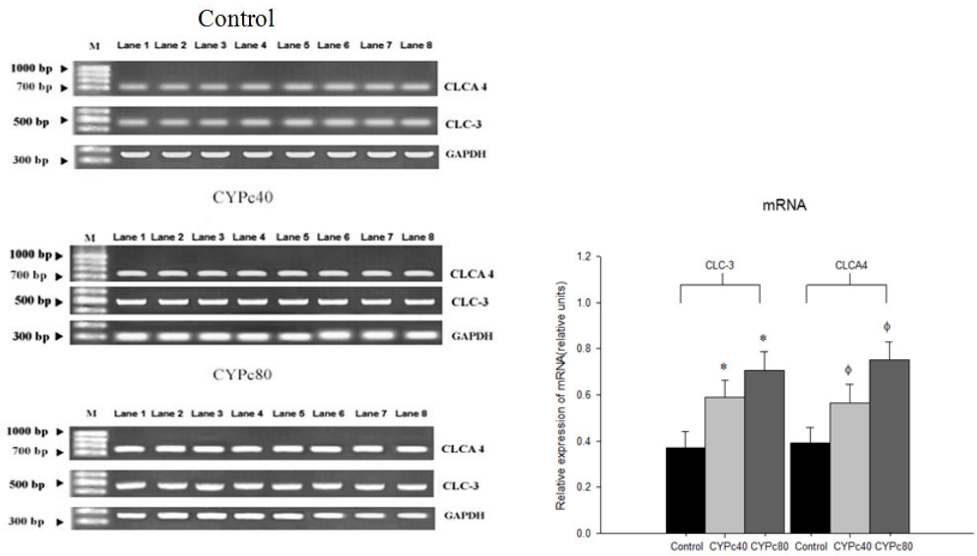
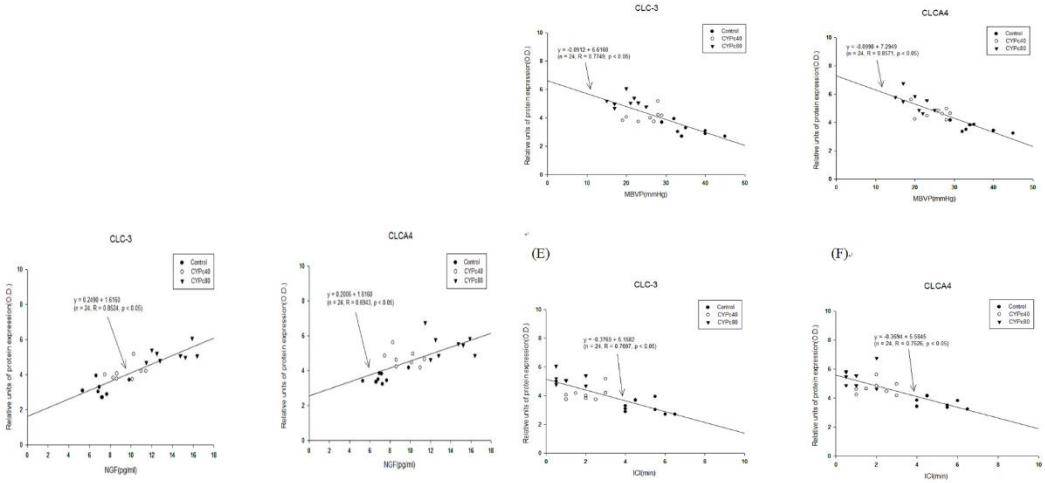


Fig. 3. Correlations of protein and mRNA expressions of chloride channels with urinary NGF and CMG parameters.



Disclosures
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