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ACID-SENSING ION CHANNEL: EXPRESSION CHANGES IN THE URINARY BLADDER FOLLOWING CHRONIC BLADDER INFLAMMATION

Hypothesis / aims of study

Cation channels of the epithelial sodium channel/degenerin family have been proposed as transducers of somatosensory stimuli in several species.(1) Since acid-sensing ion channel (ASIC) is gated by protons, it might be involved in the perception of pain during tissue acidosis. ASIC can be found in the dorsal root ganglion and many pathological conditions in peripheral tissues involve acidosis, which activates nociceptors. To date, four different genes encoding the channel, ASIC1, ASIC2, ASIC3, and ASIC4, have been cloned. We examined whether ASIC subunits are expressed in the mouse urinary bladder and how their expressions are changed following bladder inflammation.

Study design, materials and methods

The urinary bladders of female mice were harvested under deep anesthesia. To quantify the expressions of four genes (ASIC1, ASIC2, ASIC3, and ASIC4), real-time RT-PCR was performed with a Smart Cycler System using SYBR green I as the fluorogenic dye. The gene-specific primer for each ASIC was designed with the online program Primer 3. The expression was normalized as the ratio to β-actin expression. Amplified PCR products were electrophoresed on 2 % agarose gel and visualized with ethidium bromide. Some PCR products were purified and sequenced using an automated sequencing machine to identify the target gene.

Cyclophosphamide (CYP: 200 mg/kg) was injected intra-peritonealy to induce chemical cystitis. Control animals (n = 6) received vehicle treatment (i.e., intraperitoneal injection of a corresponding volume of distilled water). The expression of each ASIC gene was examined at 12 hours (n=5), 24 hours (n=5), 48 hours (n=5), 7days (n=5), 14 days (n=4) after intra-peritoneal administration of CYP.

Results

ASIC1, ASIC2 and ASIC3 genes were expressed, but ASIC 4 was virtually absent. ASIC1 and ASIC2 genes were abundant in controls. In cystitis model of mice, the expression of ASIC2 was rapidly decreased ($p < 0.01$) and gradually recovered, while ASIC1 was decreased at 12 hours ($p = 0.009$) and then increased 7days after ($p = 0.003$). ASIC3 did not show a significant change.

Interpretation of results

ASIC has attracted particular interest in the pain field because of its expression in primary sensory neurons and responsiveness to protons. It is postulated that ASIC, especially ASIC3, is involved in modulating pain sensation.(2) Experimental inflammation in the skin enhances ASIC expression in sensory neurons. The expression of some ASIC is upregulated in the inflamed intestinal mucosa. In visceral pain field, ASIC1, ASIC2 and ASIC3 has different functional roles, inhibitory or excitatory.

Cyclophosphamide-induced cystitis is often used as an animal model of visceral pain. It is known that the histological changes such as submucosal edema peaked at 12-24hours after cyclophosphamide administration.(3) In the present study, ASIC2 mRNA was significantly decreased from 12hours to 7 days after CYP injection. The response of ASIC1 expression to chemical inflammation was biphasic. ASIC2 in the mouse urinary bladder might contribute to peripheral sensitization during cystitis and nociceptive behavior.

Concluding message

We demonstrated the expressions of ASIC genes in the mouse bladder. Chemical bladder inflammation differentially altered their expression. The functional role of ASICs in the bladder remains uncertain. The present study suggested some role of ASICs in visceral sensitization by bladder inflammation. Functional experiments (e.x., in vivo cystometrograms) are needed to determine the role of ASICs in the mammalian bladder.

References
(1)Physiol Rev (2002)82: 735-767
(2)PNAS (2002)99: 8992-8997
(3)Int J Urol (2006)13: 1339-1343

Fig1
Regulation of ASIC1expression to CP injection

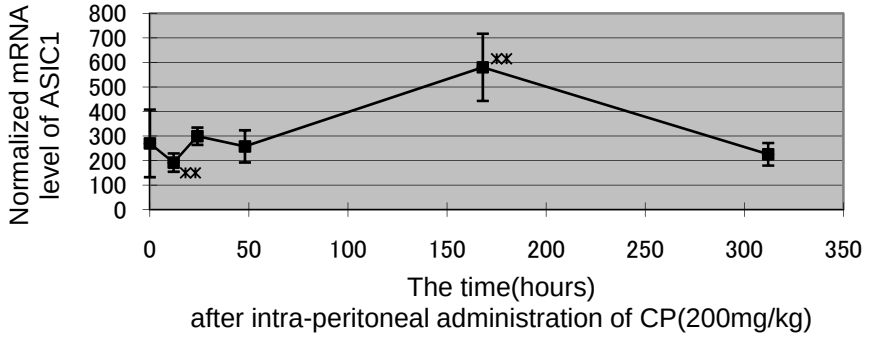


Fig2
Regulation of ASIC2 expression in response to CP injection

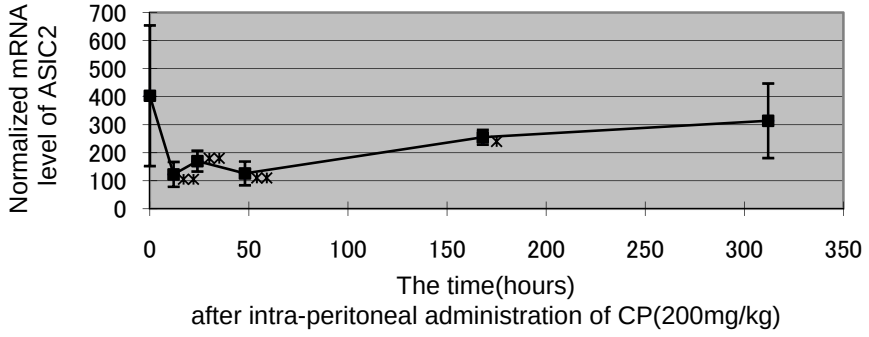
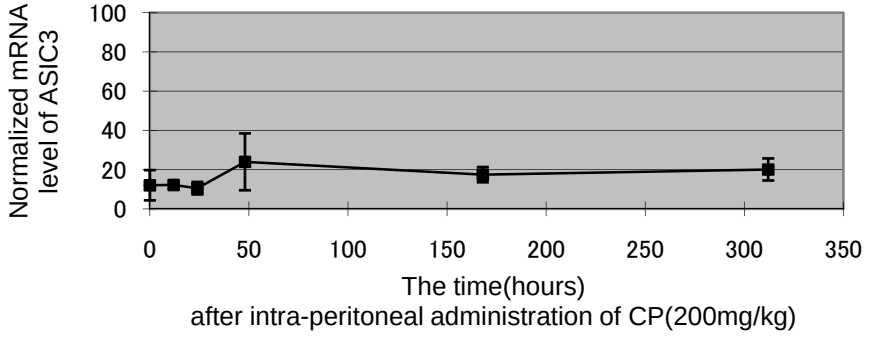


Fig3
Regulation of ASIC3 expression in response to CP injection



**p<0.01, *p<0.05

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