228

Du S[1], Araki I[1], Mikami Y[1], Zakoji H[1], Yoshiyama M[1], Beppu M[1], Takeda M[1]
1. University of Yamanashi Faculty of Medicine

THE ROLE OF EPITHELIAL SODIUM CHANNELS EXPRESSED IN THE RAT URINARY BLADDER EPITHELIUM IN MICTURITIONAL REFLEX.

Hypothesis / aims of study

The overactive bladder symptoms usually result from detrusor overactivity. Detrusor overactivity (DO) occurs in association with bladder outlet obstruction. The increase of afferent activity is one of the possible mechanisms for this idiopathic DO. Some changes in mechanosensory transduction mechanisms might be involved in this plasticity of bladder afferent activity. However, mechanosensory transduction mechanisms in the bladder afferent pathways remain to be explored.

The epithelial sodium channels (ENaC) expressed in the mammalian urothelium seem to be mechanosensitive. In the rabbit bladder, ENaC has the ability to change their sodium transport properties following changes in hydrostatic pressure. The ENaC in the pelvic epithelium of rats has been shown to participate in the activation of afferent renal mechanosensitive neurons by increased renal pelvic pressure. The ENaC was demonstrated to be up-regulated in the human bladder epithelium with outlet obstruction and instability. Thus, ENaC in the bladder epithelium might be involved in mechanosensory transduction mechanism in the bladder afferent pathways. We examined this possibility in the rat urinary bladder.

Study design, materials and methods

Immunofluorescent staining and RT-PCR:

Female Sprague-Dawley rats (10 weeks old, 200-220 g) were deeply anesthetized with sodium pentobarbital and the whole bladder was removed. Under stereoscopic microscope, the bladder mucosa was dissected from others and stored at -80 °C.

The expression and localization of ENaC proteins was examined using immunofluorescent staining. Rabbit anti-ENaC α, β and γ subunit polyclonal antibodies were used as a primary antibody. Antibody reactions were detected with TRITC-conjugated swine anti-rabbit immunoglobulin and viewed with a fluorescence microscope.

The quantification of ENaC genes expression was assessed by a real-time RT-PCR with a Smart Cycler System using SYBR green I as the fluorogenic dye. The gene-specific primers for ENaC α, β, γ subunit were designed with the online program Primer 3. The ENaC expression was normalized as the ratio (%) to GAPDH expression in each sample. 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 identified the target gene.

Cystometrograms:

Female Sprague-Dawley rats (10 weeks old, 200-220 g) were anesthetized with urethane. A transvesical catheter was used to infuse physiological saline with or without 1 mM amiloride (ENaC antagonist) and to record the bladder pressure when the bladder was continuously infused at a constant rate of 4 ml/hour. During cystometrograms, intercontraction interval (ICI), maximal voiding pressure (MVP), pressure threshold for voiding (PT) and baseline intravesical pressure (BP) were measured.

Results

The α-, β- and γ-ENaC mRNAs were found to be expressed in the urinary bladder mucosa. The expression of all ENaC subunit proteins were localized in the epithelial cells.

ICI and PT were increased by intravesically infusing 1 mM amiloride, whereas BP did not change. The effect of amiloride on ICI and PT were reversible by washing out of the drug in 6 and 4 of 8 rats tested, respectively. On the average of rats showing reversible response, the increase rate of ICI and PT by 1 mM amiloride were 47 ($n = 6$, $p = 0.02$) and 48 % ($n = 4$, $p = 0.04$), respectively.

Interpretation of results

The results clearly demonstrated that all subunits of ENaC are located in the rat urinary bladder epithelium. Cystometric parameters (intercontraction interval and pressure threshold for voiding) for afferent activity were suppressed by intravesically infused amiloride (ENaC antagonist), but the parameter (maximal voiding pressure) for efferent activity was not. Thus, the ENaC expressed in the bladder epithelium may be involved in the activation of bladder afferents.

Concluding message

The ENaC expressed in the rat bladder epithelium might play an important role in the mechanosensory transduction mechanisms in the bladder afferent pathways.

FUNDING: Japan Ministry of Education, Culture, Sports, Science and Technology, Nippon Organon K.K.