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DIFFERENTIAL EXPRESSION OF EPITHELIAL SODIUM CHANNELS IN THE HUMAN AND RAT URINARY BLADDER EPITHELIUM WITH AND WITHOUT OUTLET OBSTRUCTION.

Hypothesis / aims of study

The overactive bladder (OAB) symptoms usually result from detrusor overactivity. Detrusor overactivity (DO) occurs in association with bladder outlet obstruction (BOO). The increase of afferent activity is one of the possible mechanisms for this idiopathic DO.
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. Thus, ENaC in the bladder epithelium might be involved in mechanosensory transduction mechanism.
We examined whether the ENaC is expressed in the human and rat urinary bladder and how its expression changes in association with BOO.

Study design, materials and methods

Samples of the human bladder mucosa were obtained from 9 controls and 9 patients with BOO. The criteria for BOO included the International Prostate Symptom Score (IPSS) of more than 12 points, the prostate volume of more than 30 ml, and urodynamic tests (Qmax of less than 10 ml/sec and pressure flow study). In 7 patients with BOO, involuntary detrusor contraction was demonstrated.
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 in 5 controls and 5 rats with BOO and stored at -80 °C. To establish partial urethral obstruction, a ligature of 4-0 silk was tied around the proximal urethra with an indwelling one mm diameter polyethylene tubing under sodium pentobarbital anesthesia and then the tubing was removed three weeks before the experiment.
The expression and localization of ENaC proteins was examined using immunofluorescent staining. Rabbit anti-ENaC α, β, γ subunit polyclonal antibodies were used as a primary antibody. Antibody reactions were detected with TRITC-conjugated swine anti-rabbit immunogloblin and viewed with a fluorescence microscpore.
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 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.

Results

Immunofluorescent Staining

In the human, the α-, β-, γ-hENaC proteins were found to be expressed in the bladder epithelium with BOO, whereas the α- and γ-hENaC proteins were virtually unstained in control bladders.
In the rat, the α-, β-, γ-rENaC proteins were clearly expressed in the both bladder epithelia with and without BOO.

Quantitative Real-Time RT-PCR

In the human, the α-, β-, γ-hENaC mRNAs were detected in 1, 6 and 4 of 9 control bladder mucosa, respectively. Thus, the expression of α subunit was disproportionately low in controls. On the other hand, each hENaC mRNA was clearly present in all bladders with BOO. The expression levels of each subunit in the bladder with BOO were significantly

higher than those in controls ($p = 0.0002$ for α-subunit; $p = 0.007$ for β subunit; $p = 0.0005$ for γ subunit).
In the rat, the α-, β-, γ-rENaC mRNAs were present in all bladder mucosa with and without BOO. The expression levels of each subunit were not quantitatively different between bladders with and without BOO.

Interpretation of results

The ENaC expression in the bladder epithelium showed a remarkable species difference between the human and rat. The ENaC expression was very poor in the normal human bladder, whereas it is clearly demonstrated in the normal rat bladder.
In the human, our result indicated that BOO induces the over-expression of ENaC in the bladder epithelium. Most of patients with BOO sampled for this study showed DO. Thus, the ENac expression might be involved in the induction of DO due to BOO (idiopathic DO) in the human.

Concluding message

There is a remarkable species difference in the expression of ENaC in the bladder epithelium. In the rat, the mechanosensitive ENaC expressed in the bladder epithelium may be a good candidate for the mechanosensory transduction mechanism in the urinary bladder. In the human, the increased ENaC expression might be involved in the induction of DO due to BOO.