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# EXPRESSION OF THE THE DEGENERIN/EPITHELIAL NA+ CHANNEL (DEG/ENAC) AND THE ACID-SENSING ION CHANNEL (ASIC) FAMILY IN THE UROTHELIUM OF PATIENTS WITH SPINAL CORD INJURY AND NEUROGENIC DETRUSOR OVERACTIVITY

Hypothesis / aims of study

The Deg/ENaC family represents a new class of cation channels that was discovered at the early 1990s [1]. Recent studies on these channels have implicated them in various sensory modalities, including the lower urinary tract. Furthermore, it has been showed that the Acid-Sensing Ion Channel (ASIC) ASICs, an $H^+$-gated subgroup of the Deg/ENaC family, are involved in the physiology of mechanosensation and the pathophysiology of lower urinary tract pain [2]. Roles of each ASIC subunit in sensory function are not well understood at present.

The aim of this study is to investigate the morphological expression of these new channels in patients with spinal cord injury and to correlate the expression with the clinical conditions.

Study design, materials and methods

Specimens were obtained from normal urinary bladder (# 3 male ) and from patients with chronic spinal cord injury and urodynamic proved detrusor overactivity (# 10; 3 females, mean age 42 ± 7 years, and 7 males, mean age 39 ±11 years) by multiple cold cup biopsies during cystoscopy as the target tissue was the mucosa. Macroscopic examination of the bladder mucosa did not show any pathology at time of biopsy. All the patients gave the written consent and a local ethical committee approved the study. The morphological expression of ENaC and ASIC receptors was investigated in both the populations. Specimens were fixed in 4% PF in PBS, embedded and frozen. Slices (8mm thick) were cut by using cryostatat and incubated in the presence of the two following primary antibodies: rabbit polyclonal anti-ASIC1 (Santa Cruz Biotechnology) diluted 1:100 and goat polyclonal anti-gENAC (Santa Cruz Biotechnology) diluted 1:100. Both antibodies were applied overnight a 4°C. The immunoreactions was revealed by using the Cy2 goat anti-rabbit (Jackson ImmunoResearch) and the Cy2 donkey anti-goat (Jackson ImmunoResearch) secondary fluorescent antibodies, respectively. Both secondary antibodies were used at a final dilution of 1:333 applied for 2 h. at RT. The fluorescent immunoreaction products were observed under an epifluorescence Zeiss Axioskop microscope.

Results

In control patients, ASIC labeling is extremely faint, homogenously distributed in all the cell layers and made by few, small and scattered granules; no ENaC labelling was detectable.

In patients with spinal cord injury and detrusor overactivity ASIC labeling was uniformly distributed along the urothelium, located mainly in the more superficial layers, particularly in the club cells immediately underlining the dome-cells. The labeling is distributed along the cell profile as thin and continuous rings alternating (Fig. 1). ENaC labeling is located only in the dome-shaped cells. It appears as thin granules dispersed the cytoplasm. The labeling is uniformly distributed along the entire urothelium. The labeling intensity is lower compared to the ASIC one.

In one patient, who was refractory to oral muscarinic receptor antagonists as well as to intravesical injection of botulinum toxin A, ASIC labeling was distributed mainly along the more superficial layers, particularly in the dome-shaped cells and in the club cells immediately underlining. Interestingly, along the urothelium there are areas intensely labeled alternating to areas faintly labeled. In particular, the urothelium in the posterior wall is richer in labeled cells than the trigone. ENAC labeling intensity was lower compared to the ASIC one.

Interpretation of results

The present findings provide the evidence of the presence of ENaCs and ASICSs on human urothelium. They are overexpressed in patients with detrusor overactivity due to spinal cord injury, especially in one patients with refractory neurogenic DO. For our best knowledge, our findings are the first morphological evidence of urothelium expression of these cation channels in men with spinal cord injury and DO. They could have important implications in the pathophysiology of voiding reflex and in the mechanism of action of drugs.

Concluding message

Further functional studies remain mandatory to understand other potential roles of urothelial degenerines.

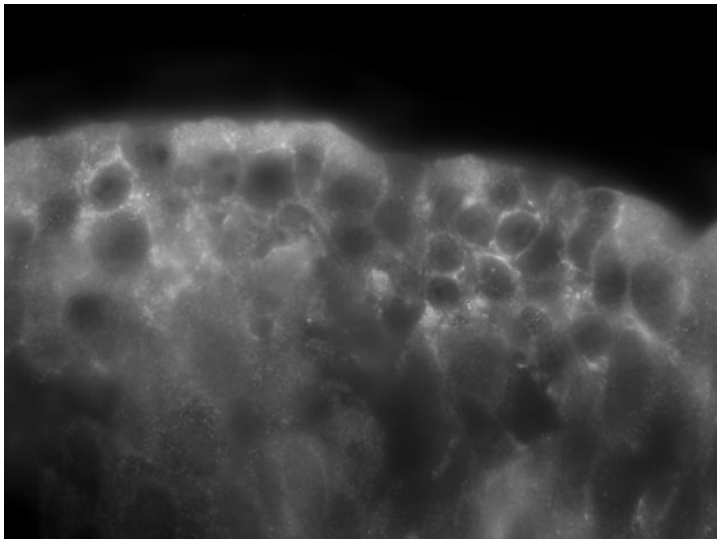

Figure 1

References

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| ***Is this a clinical trial?*** | **No** |
| ***What were the subjects in the study?*** | **HUMAN** |
| ***Was this study approved by an ethics committee?*** | **Yes** |
| ***Specify Name of Ethics Committee*** | **Comitato Etico Azienda Ospedaliero-Universitaria Careggi** |
| ***Was the Declaration of Helsinki followed?*** | **Yes** |
| ***Was informed consent obtained from the patients?*** | **Yes** |