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INTERSTITIAL CYSTITIS PRODUCE SIGNIFICANT CHANGES, WHICH COULD BE ATTRIBUTED FOR NEURONAL PLASTICITY, IN THE EXPRESSION OF PRESUMABLY SENSORY NEUROTRANSMITTERS WITHIN THE URINARY BLADDER.

Aims of Study

Interstitial cystitis is a disease, which pathogenesis and etiology are still far from being understood. Among different theories, the hypothesis of increased sensory output, as underlying cause of so-called neurogenic inflammation of the bladder, seems to be the most favorable. An increased expression of putative sensory neurotransmitters was already demonstrated in animal studies at the level of the spinal cord and the bladder wall. An increased content of some of these neurotransmitters were also found in the urinary bladder of patients with interstitial cystitis, however these results are controversial. Therefore, we aimed our study at the evaluation of the distribution pattern of possible sensory neurotransmitters in nerve fibres supplying the urinary bladder of patients suffering from interstitial cystitis.

Methods

Five female patients suffering of interstitial cystitis were included in the present study.
Interstitial cystitis was diagnosed accordingly to the criteria of the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease (NIDDK). The study protocol was approved by the Local EC Board. During the cystoscopic examination, that was performed under short general anesthesia, cold-cup bladder biopsies from the posterior bladder wall were collected. Control tissue samples (n=7) were obtained during cystectomy performed for invasive transitional cell carcinoma. Samples from patients receiving BCG therapy,patients with pathological stage > T2, as well as samples from patients with Tis were excluded from the studies. Samples were collected from unchanged region of the posterior bladder wall.
All tissue pieces were shortly fixed by immersion in freshly prepared 4% paraformaldehyde in phosphate buffer (PB; 0.1 M, pH=7.4), washed for three days in subsequent changes of PB and transferred into 18% sucrose in PB until they sank. Afterwards, 10-µm-thick cryostat sections were then stained for routine single- (or, when appropriate) double-immunofluorescence. Antisera used were polyclonal rabbit anti-CGRP (Amersham, UK; 1:4000), rabbit anti-GAL (Peninsula, UK; 1:4000), rabbit anti-VIP (Biogenesis, UK; 1:4000), rabbit anti-PACAP-27 (Peninsula, UK; 1:8000) and monoclonal rat anti-SP (Affiniti, UK; 1:350). Antigen-antiserum complex was then visualized by incubating the sections with goat anti-rabbit antiserum conjugated to biotin (Dako, Dk; 1:800) for 1 hour and, after repetitive washes, with a mixture of FITC-conjugated donkey anti-rat IgG (Jackson Labs, USA) and CY-3-conjugated streptavidin (Jackson Labs, USA). Pictures were captured by a digital camera connected with a PC, analyzed with AnalySIS software (ver. 3.2, Soft Imaging System, FRG) and printed on a wax printer (Tektronix, USA).

Results

Mean patients age was 63.8 ± 6.3 years, mean disease duration was 3.3 ± 2.1 years.
Mean age of the patients from control group was 60.6 (+8.2) years. No neoplasmatic cells were observed in the specimens studied. An increase in the expression of all examined substances, except SP and VIP, was observed in nerve fibers located under the urothelium. The highest increase was observed in the number of nerve fibers immunoreactive to PACAP-27, but not for those immunoreactive simultaneously for SP and PACAP-27.
Detailed results are presented in Table I

Table 1. Semiquantitative evaluation of the expression of various neuropeptides within and under the urothelium of the urinary bladder of control subjects and patients with IC .

Substance	Under the urothelium	
	Health	IC
SP	+	+
CGRP	+	++
SP/CGRP	+	++
GAL	±	+
SP/GAL	+	++
VIP	++	++
SP/VIP	-	+
PACAP-27	+	+++
SP/PACAP-27	+	+

± - single fibres; + - few fibres; ++ - moderate number of fibres; +++ - numerous nerve terminals.

SP – substance P; CGRP – calcitonin gene-related peptide; GAL – galanin; VIP – vasoactive intestinal polypeptide; PACAP-27 – pituitary adenylate cyclase-activating petide-27. SP/CGRP, SP/GAL, SP/VIP, SP/PACAP-27 – nerve fibers expressing immunoreactivity to both CGRP and SP, SP and GAL, SP and VIP or SP and PACAP-27, respectively.

Conclusions

Our findings demonstrate that profound changes in the expression pattern of possible sensory neuropeptides could be found in the urinary bladder of patients with IC. These changes can not only be attributed to inflammatory responses of the bladder, but also to the increased sensory output from the bladder.