131

Radziszewski P[1], Borkowski A[1], Wojtkiewicz J [2], Majewski M [2]
1. Department of Urology, Medical Academy of Warsaw, Poland, 2. Department of Physiology, University of Olsztyn, Poland

SENSORY INNERVATION OF THE HUMAN URINARY BLADDER CORRESPONDS WELL WITH THAT OF THE PIG. A MORPHOLOGICAL STUDY.

Aims of Study

Presently, there is no good animal model for studying the innervation of the human lower urinary tract in different experimentally induced pathological conditions. Well established rodent models, as well as rabbit model, are insufficient to perform for making direct extrapolation to the humans, because of many dissimilarities. These dissimilarities occurs both in the control of micturition pathway and in the occurrence, as well as distribution, of various neurotransmitters. Although to date very few studies were performed on pig model, they provided preliminary evidence that the lower urinary tract of humans and pigs has many similarities. However, to the best of our knowledge, no systemic studies on this issue were performed so far. As in the recent years more and more pathological conditions of the lower urinary tract are attributed to the sensory imbalance, we decided to compare the sensory innervation pattern of the human and pig urinary bladder.

Methods

Samples from human urinary bladder (n=7) were obtained during cystectomy performed for invasive transitional cell carcinoma. Samples from patients receiving BCG therapy and patients with pathological stage > T2, as well as samples from patients with Tis were excluded from the study. Samples were collected from unchanged region of the posterior bladder wall. Pig urinary bladder samples were obtained from n=9 sexually immature female pigs, weighing 20-30kg, after killing the animals with an overdose of sodium pentobarbital (90mg/kg). 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 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 to 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 60.6 (+8.2). No neoplasmatic cells were found in the specimens studied. Nerve fibers expressing immunoreactivity against examined substances were found both in the bladder mucosa (urothelium/submucosa) and within the detrusor muscle layer. A very high correlation in the distribution pattern of nerve fibers containing putative sensory neurotransmitters was found in bladder mucosa of human and pig. Slight differences were observed within the detrusor muscle, however the similarities were still obvious and the differences were rather of qualitative than quantitative nature.

The summary of our findings is presented in Table I

Table I. Semiquantitative evaluation of the expression pattern of various neuropeptides within the urinary bladder of human and pig

Substance	Under the urothelium/ in submucosa		Muscular coat	
	Human	Pig	Human	Pig
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

A striking similarities in the expression of the putative sensory neurotransmitters were found between human and pig urinary bladder. There is no difference in the quality and quantity in the expression of examined substances within the urothelium and submucosa. Some differences are observed within the muscular layer (rather of the quantitative nature).

We can assume that the presumably afferent innervation of the urinary bladder in humans and pigs demonstrate a pronounced similarity. Therefore, pig urinary bladder could be used as an experimental model for further studies of the pathological conditions involving altered afferent output such as e.g., interstitial cystitis.