chloral hydrate and their bladders removed and fixed for 4 hours by immersion in 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.2. After washing in buffer the bladders were cryoprotected overnight in 30% sucrose in phosphate buffer and sectioned transversely at 40 µm. Free-floating sections were immunoreacted with an antibody against the VR-1 protein (kind gift from Novartis) using the ABC-HRP method. For ultrastructural analysis the bladders of two animals were fixed for one hour in 5% acrolein in 0.1 M phosphate buffer, pH 7.2, cut in 2 mm transverse slices and post-fixed for one hour in 5% acrolein and 0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. After cryoprotection in 15% sucrose the slices were cut in a cryostat at 40 µm which were immunoreacted for the VR-1 protein as above, post-fixed with osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and observed in a Jeol electron microscope.

Results: Two distinct plexuses were evident at the light microscope. In the muscular layer numerous thin varicose fibers ran parallel to the smooth muscle fibers intimately apposed to their outer surface. Frequently, varicosities seemed to impinge on the surface of the muscle fibers. In the mucosa the majority of the immunoreactive fibers formed a loose network in close proximity to the basal cells of the transitional epithelium. Some immunoreactive fibers penetrated the epithelial layer. In the deep lamina propria VR-1-IR fibers were scarce, mostly grouped around blood vessels. No VR-1 immunoreactivity was found in epithelial or muscle cells. Under the electron microscope VR-1 immunoreactivity was found over the cell membrane and cytoplasm of unmyelinated axons and varicosities. The unmyelinated fibers were either isolated or ran together with non-immunoreactive axons in small nerve bundles. Varicosities were smooth shaped round or oval profiles in close proximity with the basement membrane of the epithelium or apposed to the membrane of muscle cells.

Conclusions: The occurrence of a large number of VR-1-IR fibers underneath the epithelium is consistent with the presumptive role of the VR-1 receptor in the transduction of chemical and thermal stimuli. Furthermore, the close proximity between VR1-IR fibers and smooth muscle cells suggests that in the bladder VR-1 may also transduce mechanical stimuli.

References: 1- J. Urol 1989; 142:150-154. 2- J Urol 1995; 154: 1825-1829. 3- Nature, 1997; 389:816-824. 4-Neuroscience, 1983; 10:861-868. 5- Neuroscience, 1986; 18: 727-747.

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Title (type in CAPITAL LETTERS, leave one blank line before the text):

IN VIVO MYOGRAPHIC STUDY IN THE PIG: URETHRA AND BLADDER RESPONSES TO PELVIC AND PUDENDAL NERVE STIMULATION

Background

An in vitro myographic model is a commonly used technique for early testing of drugs for treatment of urge incontinence.

Aims of Study

The aim of our study was to test an in vivo myographic model of the lower urinary tract in the pig. Responses to pelvic and pudendal nerve stimulation was assessed by collecting data from both the bladder and the urethra.

Methods

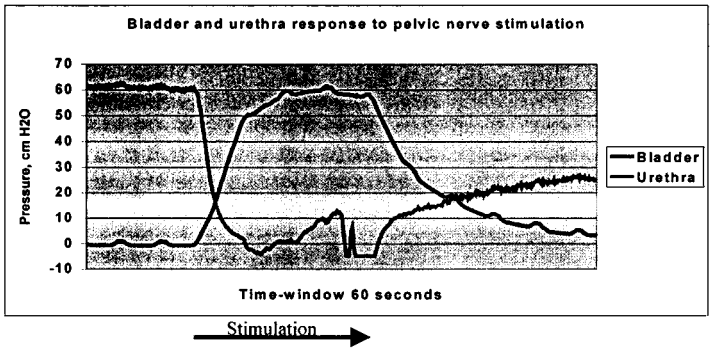
8 female pot-bellied Vietnamese minipigs median weight 44 kg (range 36-70) was used. The pigs were kept on artificial ventilation and isoflurane anaesthesia. Transurethrally an 8-french bladder catheter with three 4-french silicone-catheters attached was placed in the bladder. The balloon was pulled gently to the bladder-base leaving one eye of a pressure catheter in the bladder and 3 eyes at 1 cm intervals in the urethra. Bipolar cuff electrodes were placed on the pudendal and pelvic nerve on one side and connected to a stimulator. Square shaped stimulation pulseswere used: pulsewidth 100 μs; pulserate: 30 pulses/s, and trains of 20 s. Stimulationamplitude was 2 times threshold. Interval between stimulations on one nerve was more than 5 minutes.

The study was performed as 3 series: (1) Control; (2) α-β-methyl-ATP (Sigma)and (3) Atropine (DAK). Control-series: 4 stimulations prior to drug administration. α-β-methyl-ATP in a concentration 0,02 mg/kg was then given i.v. and 4 more stimulations were made. Atropine 0.01 mg/kg was then given iv. and further 4 stimulations were made.

Results

During pelvic nerve stimulation we consistently recorded simultaneous increase in bladder-pressure and decrease in urethra-pressure. Upon pudendal nerve stimulation we consistently recorded an increase in urethra-pressure and no change in bladder pressure. Administration of α-β-methyl-ATP 0,02 mg/kg bodyweight elicited a small but significant bladder pressure increase (n = 8, mean 4.6 cm H_2O; p <0.001). Likewise an increase in urethra pressure was recorded (n=8, mean 11.8 cm H_2O; p <0.01).

No difference in response between control-series and α-β-methyl-ATP-series was noted. In the atropine-supplement series the bladder-pressure responses was significantly lower (mean -17,1 cm H_2O, p-value <0,001 n=7) than in the α-β-methyl-ATP-series. In the urethra no significant difference in pressure-responses between the series were noted.



Conclusions

Synergy in the autonomous lower urinary tract structures seems to be organised in the periphery, incorporated in the neuromuscular transmission: pelvic nerve stimulation elicits contraction of the bladder smooth muscle and relaxation of the urethra smooth muscle. This model is capable of demonstrating both neuromuscular agonist and antagonist action of drugs acting on the bladder.

Acknowledgement

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