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THE EFFECT OF INTRAVESICAL RESINIFERATOXIN, OXYBUTYNINE, AND LIDOCAINE ON THE AFFERENT AUTONOMIC VESICAL SENSORY THRESHOLD USING A NOVEL BLADDER ELECTRODE IN RAT

Hypothesis / aims of study

Afferent bladder innervation consists of myelinated A-delta-fibers and unmyelinated C-fibers. The quantitative measurement of bladder afferent function is informative and allows a greater understanding of pathological mechanisms in lower urinary tract dysfunction. Using an intravesical electrode, we first reported the feasibility of neuroselective assessment of vesical sensory thresholds (VST) in the human bladder (1). This was accomplished with the Neurometer® (Neurotron, Inc., Baltimore, MD), a commercially available diagnostic device with neuroselective sine-wave pulse electrical stimulation (2). A recent report also found that in rats, quantitative measurement of the current perception threshold for peripheral sensation (plantar surface) using the Neurometer was feasible (3). To our knowledge, there are no reports of animal models allowing the quantitative evaluation of bladder afferent function. In this study, we developed a novel animal model for assessment of VST in conscious rats using the Neurometer®, and report the fiber-type selective effects of various intravesical agents on VST.

Study design, materials and methods

A total of 54 female Sprague-Dawley rats were used in this study. Fourteen rats underwent sham surgery, and 40 rats (Groups A, n=8; Group B, n=8; Group C, n=24) underwent implantation of our electrode in their bladder. Our bladder electrode is made of Flexon™ suture, a stainless steel twisted, multi-strand wire coated with polytetrafluoro-proethylene, and has a surface contact area with the bladder mucosa of less than 1-mm^2.

The 24-hour voiding habits of device-implanted animals (Group A, n=8) were compared to a sham group (n=14). In another 8 electrode-implanted animals (Group B), on 3 consecutive days, sine-wave electrical stimulation using the Neurometer® was applied to the bladder mucosa at increasing intensity until a light startle or vocalization response of each rat was observed. Stimulation was applied at 250 Hz and 5Hz, which has been shown to be neuroselective for A-delta and C-fibers, respectively. The minimum intensity, at which this response was seen, was defined as VST.

In the Group C (n=24), 3 days after electrode implantation, Resiniferatoxin (RTX) (1microM) (n=6), Oxybutynine chloride (0.5mg/ml) (n=6), Lidocaine (4%Lidocaine solution) (n=6), and Saline (n=6) were instilled intravesically through urethra for 30 minutes under brief general anesthesia (2% inhaled isoflurane). Conscious VST measurements were recorded prior to these administrations, and at 1 and 24hours following the instillation.

Statistical analysis was performed using Wilcoxon matched pairs signed rank sum tests and Wilcoxon sign-rank test. A $p < 0.05$ was considered significant.

Results

When comparing Group A to sham-operated rats, bladder device implantation significantly affected mean voided volume and number of voids in 24 hours (p=0.003, and p=0.006, respectively), with no significant effect on 24-hour urine output. In Group B, VST values remained constant on the consecutive 3 days of testing, with the exception of significant lower VST at 5 Hz on the post-op day 3 when compared to the post-op day 1 (p=0.016). In Group C, intravesical administrations of both Oxybutynine and Saline did not affect the VST values at either 250 or 5Hz (Table 1). 24hours after instillation of RTX, a significant increase in VST was observed at a stimulus frequency of 5Hz (p=0.046). One hour after instillation of Lidocaine, a significant increase in VST was observed at stimulus frequencies of 250Hz and 5Hz (p=0.040, and p=0.046, respectively), however, 24hours after instillation VST returned to near baseline values.

Interpretation of results

The observed startle or vocalization response in rats as a measure of VST appeared to be valid and reproducible, and allowed the determination of VSTs using fiber-type selective electrical stimulation of the bladder mucosa with the Neurometer®. This response was previously described for current perception threshold testing of peripheral sensation in rats (3). Device implantation produced a measurable effect on voiding behavior as demonstrated by a significant increase in urinary frequency. VST testing on consecutive 3 days also demonstrated a possible pathological change on C-fiber afferent, significantly lowering vesical sensory thresholds at 5Hz stimulation.

RTX, a specific C-fiber neurotoxin, caused a significant increase in VST at 5Hz stimulation, suggesting that this device could stimulate bladder afferents neuroselectively. Lidocaine-induced short-term increases in VST at 5Hz and 250Hz stimulation were thought to be the result of the non-neuroselective local anesthetic effects of the drug.

Recent studies indicated that Oxybutynine had antimuscarinic as well as local anesthetic effects, with possible effect on C-fibers also being reported. However, intravesical Oxybutynine at a clinically-used dose did not change the VST at 5Hz stimulation in our model. This may indicate limitations of this method in determining VST using the observed light startle or vocalization response.

Concluding message

In conjunction with the Neurometer®, quantitative assessment of bladder afferent function is feasible using our newly developed bladder electrode in rats. Using this animal model, we plan on assessing bladder afferent

autonomic innervations in various pathological conditions, as well as evaluating the efficacy of therapeutic agents to affect bladder sensory function.

Table 1 VST values prior to, 1 hour after, and at 24hours after intravesical administration

Agent	Frequency	Baseline VST	Post-instillation VST	
			1hour	24hours
Saline (n=6)	250Hz	26.8±4.7	28.4±4.4	24.8±7.5
	5Hz	14.4±3.4	14.8±2.4	13.0±3.1
Oxybutynine (n=6) (0.5mg/ml)	250Hz	32.7±7.0	30.0±7.9	29.7±3.6
	5Hz	13.8±4.9	14.9±5.6	13.2±6.0
Resiniferatoxin (n=6) (1microM)	250Hz	29.6±5.4	32.1±11.5	28.8±5.2
	5Hz	12.6±4.5	12.5±4.1	18.4±3.2 *
Lidocaine (n=6) (4%Lidocaine solution)	250Hz	26.6±8.6	40.5±6.4 *	27.5±6.5
	5Hz	12.5±5.3	22.9±6.8 *	14.1±5.8

Data are expressed as means ± standard deviations

*, P<0.05, statistically significant difference from baseline values using Wilcoxon sign-rank test

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(2) J Occup Med, 28: 1219, 1986
(3) J Pharmacol Exp Ther, 297: 352, 2001

FUNDING: NIH-NIDDK-R41 DK074987
DISCLOSURES: NONE
ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by Institutional animai care and use committee of Cleveland Clinic Foudation