329

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NEUROSELECTIVE STIMULATION OF AUTONOMIC AFFERENT PATHWAYS OF THE RAT BLADDER

Hypothesis / aims of study

C-fos, an immediate early gene which can be activated by increased neuronal activity, allows an accurate quantitative evaluation of nociceptive input reaching the spinal cord. Previous study demonstrated that noxious (chemical irritation) and non-noxious stimulation (bladder distension by normal saline) of the rat lower urinary tract increased c-fos expression in L6 spinal cord, including the superficial lateral and medial dorsal horn (LDH, MDH, respectively), the dorsal commissure (DCM), and the sacral parasympathetic nucleus (SPN). Noxious stimulation activated greater numbers of c-fos expression in the DCM, whereas non-noxious stimulation induced greater number of expression in the SPN(1).

The Neurometer® (Neurotron, Inc., Baltimore, MD) electrostimulator has been used worldwide to assess current perception threshold values of afferent nerve fibers, allowing clinical diagnosis of hyperesthesia or hypoesthesia in various peripheral neuropathies(2). The Neurometer® can deliver stimuli at frequencies of 5, 250 and 2000 Hz, which have been reported to selectively stimulate C, Aδ and large myelinated (Aβ) fibers, respectively. Recently, we developed the Vesical Sensory Threshold electrode device (VST), which is implanted on the bladder mucosa in rats and used with the Neurometer® to assess bladder afferent nerve function(3).

In this study, we aimed to compare the quantitative expression of the c-fos in response to incremental neuroselective stimulation of C-fibers versus A-delta and A-beta-fibers of the bladder.

Study design, materials and methods

Thirty-three female Sprague-Dawley rats were used in the experiments. The rats were divided into seven groups: 5-Hz stimulation with 1.5 mA (n = 4) or 2.0 mA (n = 6); 250-Hz stimulation with 1.5 mA (n = 4) or 2.0 mA (n = 6); 2000-Hz stimulation with 1.5 mA (n = 4) or 2.0 mA (n = 4), and Control(n=5).

Electrode implantation and electrical stimulation: Under general anesthesia (urethane 1.2 g/kg), the electrode was implanted in the posterior bladder. Immediately following implantation, electrical stimulation was applied via the Neurometer® to the bladder for 90 min at 2000 Hz, 250 Hz or 5 Hz. In the control group, electrode implantation was done similarly, but no electrical stimulation was applied.

Expression of c-Fos: After sacrifice, spinal cord sections were checked for immunoreactivity to c-Fos protein, and c-Fos-positive cells in the spinal regions of the medial dorsal horn, lateral dorsal horn, dorsal commissure (DCM), and sacral parasympathetic nucleus (SPN) were measured. Total and regional distributions of positive c-Fos neurons were compared between the groups. The Mann-Whitney U test was used to analyze differences, and $p < 0.05$ was considered significant.

Results

Compared to electrode implantation alone, 5 Hz stimulation increased c-Fos expression significantly at 1.5 and 2.0 mA intensities, while 250 Hz stimulation increased c-Fos expression significantly only at the relatively high 2.0 mA intensity(Figure 1). 2000 Hz stimulation did not increase c-Fos expression significantly beyond electrode implantation alone at either intensity(Figure 1).

After stimulation of the bladder at 5 Hz and 250 Hz, we calculated the percentages of c-Fos positive cells in each of the four regions of L6 spinal cord found to contain positive cells (MDH, LDH, DCM, SPN). 5 Hz stimulation at 1.5 and 2.0 mA induced the highest amount of c-Fos expression in the DCM, while 250 Hz (2.0 mA) stimulation induced the highest c-Fos expression in the SPN(Figure 2).

Interpretation of results

We stimulated bladder afferent fibers with various intensities of sine-wave electrical stimulation and assessed the expression of c-Fos. Our data correspond well with a previous report indicating that non-nociceptive stimulation of the bladder (distension with saline solution), thought to stimulate Aδ fibers, induced c-Fos expression predominantly in the SPN region of L6 spinal cord, while nociceptive stimulation (1% acetic acid), shown to stimulate C fibers, induced the most c-Fos expression in the DCM(1). Our results demonstrate that neuroselective sine-wave electro-stimulation of the bladder is a feasible method by which we can evaluate the function of bladder afferent nerve fibers.

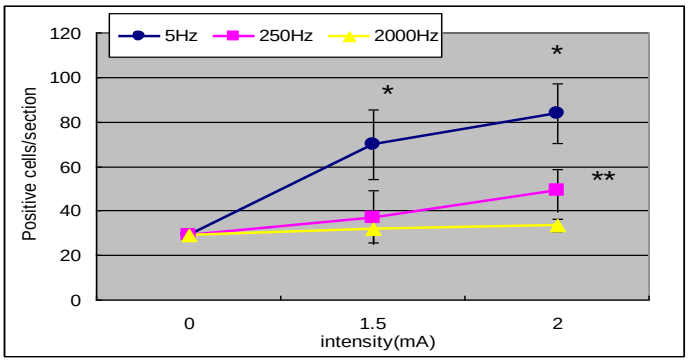
Concluding message

Sine-wave electrical stimulation of the rat bladder increased c-Fos expression in a neuro-selective manner. Thus, our newly developed model could be used for studies of disturbances of afferent pathways of the bladder.

References

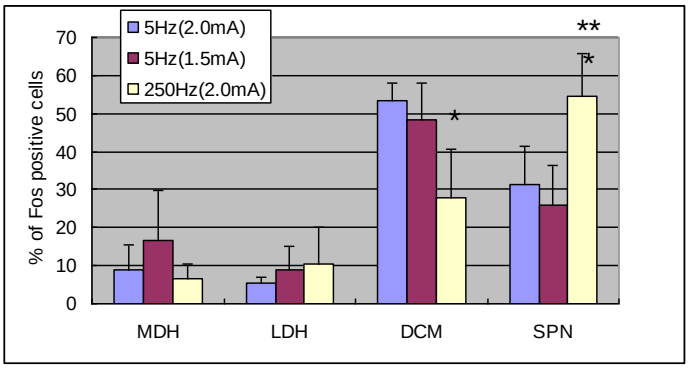
(1) J Neurosci 1992; 12: 4878
(2) J Occup Med 1986,28: 1219
(3) J Urol 2008; 179: 1167

Figure 1: Histogram showing c-Fos-positive cells per section at L6 following electrical stimulation (at 5, 250, and 2000 Hz).



*Achieved statistical significance ($p < 0.01$) between 5-Hz stimulated (1.5 mA or 2.0 mA) group and unstimulated (0 mA) control group.
**Achieved statistical significance ($p < 0.01$) between 250 Hz stimulated (2.0 mA) group and unstimulated (0 mA) control group.

Figure 2: Histogram showing the distribution of c-Fos positive cells in four regions of the L6 spinal cord following electrical stimulation.



*Achieved statistical significance ($p < 0.01$) between 5-Hz (2.0 mA) and 250-Hz (2.0 mA) stimulated groups.
**Achieved statistical significance ($p < 0.01$) between 5-Hz (1.5 mA) and 250-Hz (2.0 mA) stimulated groups.

Specify source of funding or grant	**NIH**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional animal care and use committee of the Cleveland Clinic**