522

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THE EFFECTS OF BILATERAL BIPOLAR L6-S1 TRUNK NEUROSTIMULATION ON CONTINUOUS CYSTOMETRIC PARAMETERS BEFORE AND AFTER BLADDER IRRITATION

Hypothesis / aims of study
Sacral neurostimulation has been found to be effective in the treatment of overactive bladder and urge incontinence, and has been in use clinically for over a decade. However, the precise mechanisms by which this positive effect on symptoms occurs remain unknown, although pharmacological studies are beginning to shed some light. Further understanding of the mechanism of action requires a robust experimental model.

With the exception of one study, previous research directed toward understanding the effects of sacral neurostimulation on lower urinary tract activity in the rat has utilized blind placement of unipolar electrodes into the S1 foramen, relying on stimulus feedback for final positioning. The exception to this approach was that of bilateral monopolar stimulation of the isolated L6 roots. Functional urologic outcomes in these prior studies of neurostimulation in spinal cord intact rats have included metabolic cage void collections, limited single fill cystometry and isovolumetric studies, with little data available on the effect of sacral neurostimulation on non-voiding contractions (NVC) during filling.

Prior investigation has revealed the presence of base-to-dome NVC of myogenic origin that are associated with normal filling in the rat. In addition, apparently reflex dome-to-base NVC in response to the filling contractions were observed. It is important to determine whether there is a differential effect of sacral neurostimulation on these two NVC types. The current study was designed to determine the effect of bilateral bipolar L6-S1 trunk neurostimulation on lower urinary tract function, as determined by continuous cystometry, in anesthetized female rats before and after intraluminal irritation.

Study design, materials and methods
Female Sprague-Dawley rats (n=10; 244-372 g body weight) were anesthetized with urethane (1.2 g/kg) and the bladders were exposed by a midline laparotomy. A normal saline-filled PE50 catheter with a heat-flared tip was inserted through a cystotomy in the apex of the dome and was secured by ligature which included the margins of the bladder wall. The abdomen was closed in layers, the animal was repositioned in a ventral recumbency and a midline incision was made in the skin overlying the sacrum.

The lumbosacral and L6-S1 trunks were exposed and the L6-S1 trunk was dissected from surrounding tissues and isolated with a small sheet of parafilm, thereby isolating the L6-S1 trunks electrically in that region. Electrodes fashioned from 50 um diameter stainless steel wire with epoxy coating (M.T. Giken Co., Tokyo, Japan) were soldered to insulated copper wire leads. Rostral poles were positioned rostrally on the nerve bilaterally, and the caudal poles were positioned caudally on the nerve bilaterally. The trunks were covered with mineral oil and the wires were anchored into place on midline tissues using tissue adhesive. The back skin was carefully closed with wound clips.

The animals were placed on a heating pad and the bladder catheter was connected to an infusion pump and pressure transducer by 4-way stopcock and the electrode leads were paired as poles and joined at the binding post of the FHC Pulsar 6bp stimulator terminals, with the rostral poles inserted into the output post. Cystometric tracings were recorded using ADInstruments LabChart 7. Continuous cystometry was performed with room temperature normal saline at an infusion flow rate of 0.1 ml/min. Neurostimulation was performed at 10 Hz, 0.1 msec pulse duration, current directions of positive, negative or bipolar at below threshold intensities for tail and limb movement (ranged from 0.15 to 0.80 mA). Following neurostimulation testing in the control (unirritated) state, the infusate of half of the animals was switched to 0.25% acetic acid in saline to induce bladder irritation. Neurostimulation efficacy was then also tested under irritated conditions. Measured outcomes included bladder capacity, filling compliance, and NVC count, maximum amplitude, mean amplitude and period. By using a band-pass filter with a high cut off frequency of 0.1 Hz and a low cut off frequency of 0.01 Hz (bladder data collected at 1k/s), we were able to represent NVC activity in a format that allowed for cyclic measurement peak detection, thus enabling us to make NVC counts and mean amplitude and cycle period estimates. Maximum NVC amplitude measurements were made directly. Pre- and post-neurostimulation data were compared using paired t test, unirritated vs. irritated using Mann-Whitney and the relationships between NVC parameters and bladder capacity were investigated using linear regression. GraphPad Prism 6 was used to analyse statistics and $P<0.05$ was considered significant.

Results
Mean bladder capacity was more than doubled (P=0.0009) and quintupled (P=0.0272) by neurostimulation under unirritated and irritated conditions, respectively. Acetic acid infusion resulted in a reduction to 19% of control bladder capacity (P=0.0007). In four preparations, neurostimulation resulted in a complete abolition of the micturition reflex and overflow incontinence under control conditions and, in two of these animals, also under irritated conditions. Filling compliance was unaffected by neurostimulation regardless of infusate condition, but was decreased to 31% of control by acetic acid irritation (P=0.0308). NVC count was increased to 192% (P=0.0023) and 638% (P=0.0316) of pre-stimulation counts by neurostimulation under unirritated and irritated conditions, respectively. Acetic acid infusion resulted in a reduction to 12% of control counts (P=0.0007). Linear regression revealed a significant (P=0.0013) positive relationship between NVC count and bladder capacity. Maximum NVC

amplitudes were increased to 200% (P=0.0161) and 267% (P=0.0490) by neurostimulation under unirritated and irritated conditions, respectively. No significant effect of acetic acid on maximum NVC amplitude was observed. Ranges of maximum NVC amplitude were 0.42 and 6.2 cm H2O and 0.50 and 8.53 cm H2O under unirritated and irritated conditions, respectively. Mean NVC height was not different for any comparison and NVC period was unaffected by neurostimulation.

Interpretation of results

Bladder capacity was enhanced in both the unirritated and irritated conditions, with a reversal of acetic acid-induced reduction by neurostimulation. Interestingly, filling compliance was unaffected by neurostimulation, although reduced by acetic acid. This finding may suggest a local effect of irritation on compliance, while the reduction in bladder capacity by irritation and its reversal by neurostimulation is most likely attributable to changes in afferent input to the central nervous system. The increase in NVC count due to neurostimulation can be attributed directly to an increase in bladder capacity, as is also likely the case for maximum NVC amplitude. It is important in this regard to note that the maximal NVC amplitude recorded was below 10 cm H2O, a generally accepted limit of physiological significance – thus, we interpret these NVC to all fall within the category of myogenic base-to-dome filling contractions. That mean NVC height and period were not changed by neurostimulation also supports the contention that these NVC were reflections of myogenic activity rather than under nervous control.

Concluding message

We have demonstrated that bilateral bipolar stimulation of the L6-S1 trunks is capable of increasing bladder capacity without affecting normal myogenic motor activity associated with filling. That such stimulation is capable of completely abolishing the micturition reflex in some animals with or without intraluminal irritation demonstrates the utility of this model for future pharmacologic dissection of the mechanism of action for this therapeutic approach.

Disclosures

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