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Salvadeo D[1], Mazzei M[1], Tiwari E[2], Roberts A[1], Frara N[1], Braverman A[1], Barbe M[1], Ruggieri M[1]
1. *Lewis Katz School of Medicine at Temple University,* ***2.*** *Electrical and Computer Engineering Temple University*

CONTRACTILITY OF BLADDER SMOOTH MUSCLE IS PRESERVED AFTER LOWER SPINAL CORD INJURY

Hypothesis / aims of study

Denervation causes rapid loss of skeletal muscle mass and contractility. While skeletal muscle degeneration following nerve injury has been well investigated, comparably little is known about the effects of nerve root injury on smooth muscle. We studied changes in nerve and smooth muscle function of the bladder after sacral root transection to confirm the preservation of smooth muscle contractility despite the loss of presynaptic input.

Study design, materials and methods

We designed a lower spinal cord injury model in which animals underwent surgical transection of all sacral roots, eliminating a majority of motor input to the bladder. Sham-operated controls received a lumbosacral laminectomy without root transection. Animals were housed for a year following the initial surgery. Three weeks prior to sacrifice, bladders were cystoscopically injected with a neuronal retrograde label at four positions around the ureterovesical junction for later pelvic plexus ganglia examination. During terminal surgery at 1 year post sacral root transection, pelvic plexus nerves adjacent to the bladder were stimulated with either a monopolar or bipolar probe using a current of 3 - 6 mAmp, a frequency of 20 Hz, and a duration of 0.2 msec. Contractions were recorded with external pressure transducers interfaced with the PowerLab® multichannel data acquisition system and LabChart® software (ADInstruments, Colorado Springs, CO). Following euthanasia, strips of smooth muscle were isolated from bladders and denuded of the mucosal layer. Electric field stimulation was delivered to the muscle strips, *ex vivo*, at 12 V, with a duration of 1 ms, at varying frequencies using a Grass S88 stimulator (Natus Neurology Inc., Warwich, RI) interfaced with a Stimu-Splitter II (Med-Lab Instruments, Loveland, CO) power amplifier and LabChart® software (ADInstruments, Colorado Springs, CO). Also following euthanasia, pelvic plexus and bladder tissues were collected, fixed in 4% buffered paraformaldehyde and cryosectioned. Bladder tissues were immunostained with anti-caspase 3 (diluted 1:20, Millipore, Billerica, MA). Sections were counterstained with a nuclear stain before cover slipping with 80% glycerol in PBS for visualization and quantification of caspase immunostaining. The external urethral sphincter was also harvested and immunostained for caspase 3 to confirm its denervation.

Results

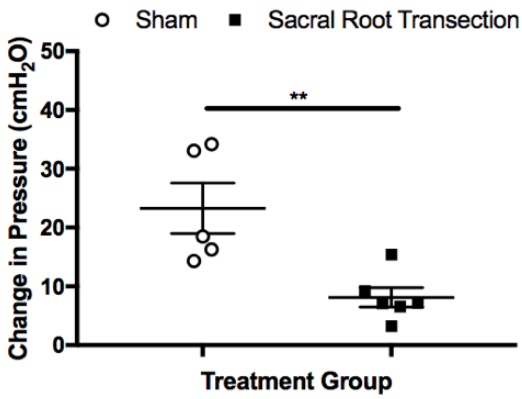

Figure 1. Bladder contractility via stimulation of the pelvic plexus decreases after long-term decentralization *in vivo*. Each point represents the maximal contraction yielded from stimulation of either left or right pelvic plexus of a single animal, whichever produced the strongest bladder contraction. Animals that received sacral root transection (black, n=6) had a significant decrease ($p<0.01$) in maximal bladder contraction after pelvic plexus stimulation compared to sham-operated controls (white, n=5).

The pelvic plexus maintained its ability to induce bladder contractions a year after spinal cord injury (Fig. 1), tested *in vivo*, although sacral root transection significantly decreased maximal bladder contraction ($p<0.01$).

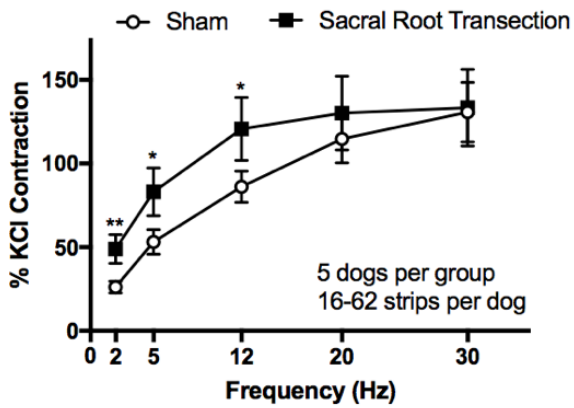

Figure 2. Nerve-evoked bladder smooth muscle contractility increases *ex vivo*. Muscle strips were exposed to KCl prior to electric field stimulation. Frequency-response curves were generated by exposing strips to varying frequencies while keeping voltage (12 V) and duration (1 ms) consistent. Contractions were measured as a percentage of 120 mM KCl-induced contraction. Smooth muscle strips from animals that received sacral root transection (black) generated a force of contraction greater than strips from sham-operated controls (white) at 2 Hz ($p<0.01$), 5 Hz ($p<0.05$), and 12 Hz ($p<0.05$). The change in slope regarding frequencies significantly differed between groups ($p<0.05$).

Interestingly, prior sacral root transection significantly altered the change in slope of the frequency response-curve in isolated smooth muscle strips *ex vivo* ($p<0.05$). The injury also caused an increase contraction in response to stimulation frequencies of 2 Hz ($p<0.01$), 5 Hz ($p<0.05$), and 12 Hz($p<0.05$), compared to sham-operated controls.
No difference was seen in the number of pelvic plexus neurons labelled with retrograde dye between the sham-operated group (8.96 ± 2.97 cells/mm^2, n=5) versus the sacral root transection group (14.57 ± 5.565 cells/mm^2, n=3) in a previous cohort of dogs. Likewise, there was no difference in caspase 3 immunostaining in the smooth muscle of the bladder between groups. Thus, surgical transection of the sacral roots did not increase levels of apoptosis in detrusor muscle. In contrast, and as expected, striated muscle in the external urethral sphincter (EUS) of sacral root transected dogs (n=6) showed positive caspase 3 immunostaining, while no caspase 3 immunostaining was seen in EUS of sham-operated controls (n=5). Striated muscle fascicles of EUS in the injury model appeared atrophied and exhibited centronucleation, indicative of cell death, pathology not observed in sham animals.

Interpretation of results

The decrease in pelvic plexus stimulation-induced bladder contractions *in vivo* suggest that long term loss of presynaptic input from the spinal cord directly effects the function of nerves in the pelvic plexus and bladder wall. Differences in results from electric field stimulation *ex vivo* indicate a change in phenotype of nerves found in the intramural wall of the bladder at one year post sacral root transection. It is possible that the root injury induced nerve sprouting. However, despite the decrease of neuronal input, no degeneration of bladder detrusor muscle was observed. This, combined with the preservation of smooth muscle contractility, may indicate that detrusor smooth muscle integrity does not depend on external neuronal input. Alternatively, other neuronal inputs (originating from sites other than the sacral cord) may be maintaining the detrusor smooth muscle.

Concluding message

Smooth muscle cells within the bladder maintain function after lower spinal cord injury. Although pelvic plexus-induced bladder contractions were less robust at one year after sacral root transection, the absence of atrophy and preservation of at least some nerve activity may allow for successful surgical reinnervation after long-term injury.

Disclosures

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