501:Neuromuscular nicotinic receptors mediate upper lumbar and lower thoracic spinal root stimulation-induced bladder contractions in canines

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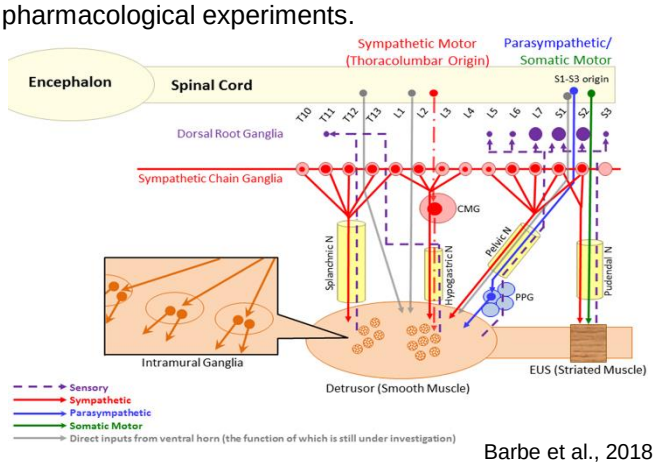
INTRODUCTION

Micturition and urine storage are complex activities primarily driven by parasympathetic innervation from the sacral spinal cord and sympathetic innervation from the lower thoracic and upper lumbar spinal cord respectively. Additionally, we previously found that the detrusor is also innervated by a small number of direct inputs originating in the lower thoracic and upper lumbar ventral horns, the function of which is not yet understood. To characterize these direct fibers, we conducted in vivo and ex vivo pharmacological experiments.



Barbe et al., 2018
The Anatomical Record

METHODS

Three female mixed-breed hounds underwent a laminectomy from spinal level T10 to S4. Roots originating from each level were stimulated and changes in pressure were recorded. Sacral roots and hypogastric nerves were then transected bilaterally. Neuromuscular nicotinic receptor antagonist atracurium was administered at 25 mg/kg and lower thoracic/upper lumbar roots were stimulated. Data were analyzed using an unpaired t-test. At euthanasia, the bladder was harvested and strips of smooth muscle devoid of mucosa were isolated from bladders. The contractile response to electric field stimulation was determined in the presence of 5 µM atracurium (n=18-24 strips from 5 animals). Data were analyzed using 2-way ANOVA followed by Tukey's multiple comparisons test. Remaining bladder tissue was stored for future assays.

RESULTS

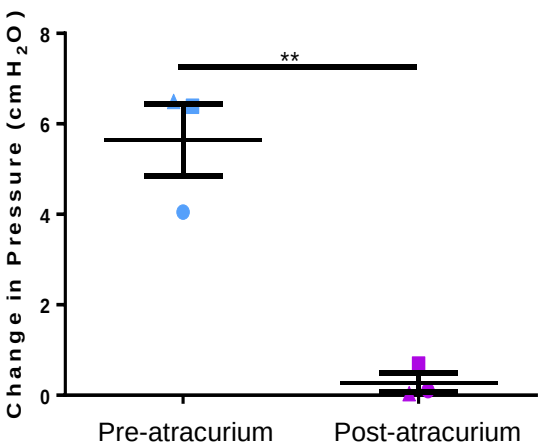


Figure 1. IV administration of 25 mg/kg atracurium significantly decreases L2 root stimulation-induced detrusor contraction. Pre-drug stimulation represents the maximal bladder contraction generated by L2 root stimulation prior to drug administration. Post-drug stimulation shows the effect of atracurium on L2-mediated bladder contractions. Treatment with atracurium significantly decreases L2 root stimulation-induced bladder contractions (n=3; p<0.005).

RESULTS

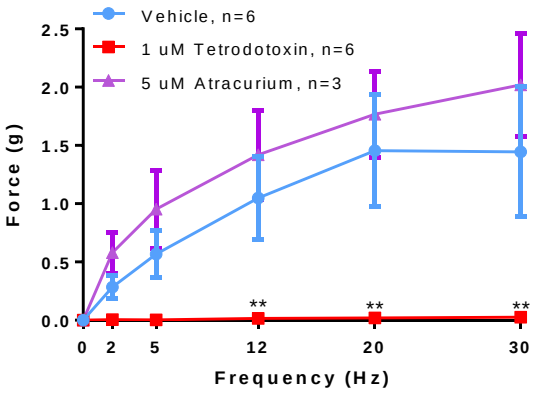


Figure 2. Treatment of 5 µM atracurium does not block EFS-induced contraction of bladder smooth muscle ex vivo. Isolated smooth muscle strips were treated with 5 µM atracurium prior to receiving EFS. Atracurium did not have an effect of the strength of contraction at any tested frequency. Separate strips were also treated with 1 µM tetrodotoxin (TTX), which significantly blocked EFS-induced contractions at all frequencies, confirming that the contractions are mediated by nerves.

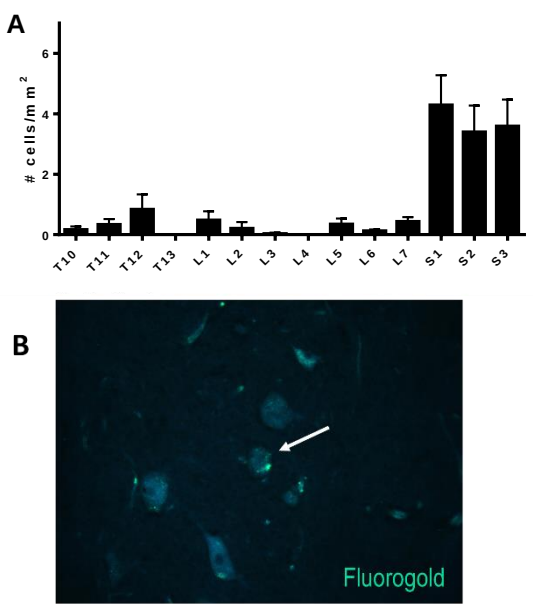


Figure 3. Bladder also receives innervation from the thoracolumbar spinal cord. A. Count of Fluorogold-positive cells found in the ventral horn of the spinal cord after cystoscopic injection of neuronal retrograde label. While the majority of positive cells are seen in the sacral spinal cord (as expected), positive cells were also identified in levels T10-T12, L1-L2, and L5-L7. Positively labeled cells in the ventral horn suggest direct fibers from the spinal cord to the wall of the bladder. B. Image of Fluorogold-positive cell bodies in the spinal cord. Positive cell indicated with a white arrow.

CONCLUSIONS

The presence of detrusor contractions after lower thoracic/upper lumbar root stimulation regardless of sacral root and hypogastric nerve transection suggests that the contractions are mediated by nerves other than the traditional sacral parasympathetic or hypogastric sympathetic innervation. A significant decrease in detrusor contraction after treatment with atracurium indicates that activity of this subpopulation of nerves is mediated by neuromuscular nicotinic receptors. Furthermore, the nicotinic receptors are not located in the intramural ganglia due to the absence of change in contractility of isolated bladder smooth muscle after treatment with atracurium ex vivo. Functional motor innervation of the bladder from above the lumbosacral spinal cord may provide new options for treating urinary incontinence following lower spinal cord injury.