occurs after SCI. These neuroplastic processes may relate to remodeling of the end organs of the bladder and the urethra; however the exact changes and patterns of neuroplasticity after SCI remain unknown. This study aims to identify micturition reflex network at lumbosacral and bladder dysfunction after T8 complete SCI using pseudoabies virus (PRV) transneuronal tracing technology.

METHODS: Eighty-five adult Sprague-Dawley female rats (225-275g) were used. All rats were divided into 3 groups: naive control, Tx-15 and Tx-45 (15 days and 45 days survival period after complete SCI procedure at the level of T8, respectively). Each group was further subdivided into 2 groups according to PRV injection to bladder body or external urethral sphincter (EUS). Three days after PRV injection to bladder or EUS, animals underwent perfusion followed by tissue harvest of bladder, urethra, and spinal cord. Thirty μm transverse sections were collected from the middle of L4 to the middle of S3 spinal segment for each sample. Animals in Tx-45 group also underwent urodynamic study 3 days before PRV injection.

RESULTS: Bladder remodeling occurs and becomes exacerbated 15 and 45 days after SCI according to the statistically significant changes of bladder weight. Bladder weight mirrors the bladder dysfunction. The main PRV-positive spinal segment from bladder injection of PRV after SCI moves distal with time from L6-S1 to S1-S2. The number of PRV-labeled neurons was positively correlated with bladder weight 15 days after bladder injection and 45 days after EUS injection, suggesting neuroplasticity after SCI occurs earlier in the pathway involving the bladder than in the pathway involving the urethra.

CONCLUSIONS: The window of time between bladder and urethra neuroplasticity after T8 complete SCI may indicate the time for full completion of micturition control pathway reorganization at the lumbosacral level. Further studies using specific synaptic stains in the spinal cord will be used to determine the synaptic plasticity and reorganization of the micturition reflex pathway after lumbosacral level SCI.

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MYOSIN-VA PROMOTES PURINERGIC NEUROTRANSMISSION IN THE BLADDER

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INTRODUCTION AND OBJECTIVES: Myosin motor proteins play a crucial role in smooth muscle neurotransmission by translocating secretory vesicles containing neurotransmitters to the membrane of varicosities, where exocytosis occurs. Myosin Va in particular appears to be essential for enteric neurotransmission since previous studies showed that myosin-Va deficient (DBA) mice exhibit impaired purinergic inhibitory junction potentials in the gut. In the bladder, excitatory neurotransmission is predominantly accomplished by release of acetylcholine and ATP from varicosities; however little is known about the molecular mechanisms underlying vesicle exocytosis. In this study, we investigated the contribution of myosin-Va to neuromuscular transmission in the bladder.

METHODS: Bladder tissue was procured from DBA mice. Wild type C57BL/6J (WT) mice bladders were used as control. Longitudinal strips were stretched in organ bath under 0.5 grams of tension at 37°C. Neurally evoked contractile responses were generated by electrical field stimulation (EFS) delivered in the presence or absence of muscarinic receptor antagonist atropine, or purinergic receptor antagonists NF449 and BDBD. Contractile responses to exogenous carbachol (CCh), α-β-methylene ATP ($\alpha\beta$meATP) or KCl were also measured. Myosin-Va distribution in bladder tissue was investigated by confocal microscopy.

RESULTS: Under baseline conditions, contractions induced by EFS were significantly lower in DBA compared to WT bladder tissue. The administration of NF449 and BDBD significantly decreased the amplitude of EFS-induced contractions in bladders from WT but had no effect on electrically induced contractions in DBA bladders. In DBA, the atropine-resistant component of EFS-induced bladder contractions was significantly lower compared to WT. No differences between strains were detected in the amplitude of responses to exogenous CCh, $\alpha\beta$meATP or KCl. Positive immunoreactivity for Myosin-Va was detected on nerve fibers within bladder tissue.

CONCLUSIONS: These findings show that the purinergic component of neurally mediated detrusor contractions is impaired in myosin-Va deficient mice due to a prejunctional defect, since the contractile response to exogenous $\alpha\beta$meATP was not different between strains. These data suggest that myosin-Va plays a major role in driving the contractile response to purinergic neurotransmission by transporting the ATP-containing vesicles to the membrane of varicosities.

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ROLE OF CANNABINOID AND TRPV1 RECEPTORS IN THE SPINAL MECHANISMS OF MICTURITION IN THE NORMAL RAT

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INTRODUCTION AND OBJECTIVES: Systemic administration of cannabinoid (CB) receptor agonists and fatty acid amide hydrolase (FAAH) inhibitors affects bladder function, but whether the main site of action is peripheral tissues or the central nervous system is unknown. Intrathecal (IT) CBs have been shown to produce antinociception in neuropathic pain animal models. Many of these compounds also act at the TRPV1 receptor. Our goal was to determine the effects of IT CB receptor agonists and the impact of TRPV1 activation on bladder function of normal rats when spinal degradation of endogenous CBs is inhibited.

METHODS: In female rats, bladder and IT catheters were inserted prior to cystometric evaluation. Urodynamic parameters were recorded in conscious animals at baseline, and after each drug. The first part of the study involved two groups of animals with incremental doses, preceded by vehicle, of methanandamide (MA) (5, 10, 20, 40 μg), a selective CB1 agonist and WIN 55212-2 (10, 20, 40 μg), a non-selective CB agonist. The second part involved two other groups of animals: the first received IT SB366791 (200 nmol), a selective TRPV1 antagonist, followed by intraperitoneal oleoyl ethyl amide (OeTA) (0.75 mg/kg), a FAAH inhibitor, and finally IT MA (100 μg); the second group received IT vehicle (DMSO) for SB366791, followed by OeTA, and IT MA. Parameters were measured in absolute values and calculated as change from baseline.

RESULTS: The micturition pressures did not change after vehicle or drug administration across all groups. In the MA only group, bladder capacity (BC) significantly increased from baseline after 40 μg administration (0.62 vs 0.91 mL, $p<0.05$). BC also significantly increased after administration of 40 μg of WIN 55212-2 when compared to baseline (0.80 vs 1.04 mL, $p<0.01$) and to vehicle (0.82 vs 1.04 mL, $p<0.05$). Micturition volume (MV) increased from baseline after 20 μg administration of WIN 55212-2 (0.76 vs 1.05 mL, $p<0.05$). In the animals that received vehicle prior to OeTA and MA, we observed a significant increase from baseline in the BC (13.9%) and MV (31.8%) following both systemic OeTA and IT MA ($p<0.05$). While in the animals that were given SB366791, there were no significant changes from baseline in BC or MV following systemic OeTA and IT MA.

CONCLUSIONS: IT CB receptor agonist administration increases BC in normal rats. Both drugs may activate TRPV1 along with CB receptors. IT TRPV1 antagonist administration abolished the effects of both MA and OeTA on BC and MV. This suggests that spinal TRPV1 activation is involved in effects by FAAH substrates and MA on afferent signaling in micturition.

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