POSTER SESSION 1

P1.1. ANTINOCICEPTIVE EFFECT OF BOTULINUM TOXIN TYPE A ON PERSISTENT BILATERAL ALLODYNIA INDUCED BY INTRAMUSCULAR CARRAGEENAN IN RATS

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Introduction: In the present study we characterize the localization of antinociceptive effect of botulinum toxin type A (BT-A) in "mirror pain" (MP) induced by intramuscular -carrageenan injection. Additionally, we investigate the role of spinal glial cells in MP and in the effect of BT-A.

Materials and methods: 100 μL 3% carrageenan (dissolved in saline) or saline was injected into right gastrocnemius muscle of male Wistar rats. Animals which developed bilateral mechanical allodynia two weeks following carrageenan injection were divided into following groups: (1) and (2) saline or BT-A (5 U/kg) subcutaneously into the right hind-paw pad, (3) BT-A (5 U/kg) subcutaneously into the left hind-paw pad, (4) and (5) saline or BT-A (1 U/kg) in the spinal canal, (5) and (6) saline or BT-A (1 U/kg) into the cisterna magna. Nociceptive measurements were performed using von Frey filaments. Glial activation markers (GFAP and CD11b) in the lumbar spinal cord were assessed by immunohistochemistry.

Results: Ipsilateral BT-A, 5 days following application, decreased mechanical hypersensitivity on both hind paws ($p<0.05$): ipsilateral and contralateral to injury. Similarly, intrathecal BT-A exerted the bilateral antinociceptive effect ($p<0.01$), but after 1 day. In contrast, contralateral BT-A treatment and the application in cisterna magna had no effect on pain on either tested side. Astrocytes and microglia were not activated in this type of persistent pain.

Conclusion: Because BT-A's bilateral antinociceptive effect can be achieved after peripheral ipsilateral and intrathecal, but not intracisternal and contralateral application, its effect on MP apparently occurs at segmental spinal level and does not involve spinal glia.

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P1.2. TETANUS TOXIN-INDUCED RAT MODEL OF FOCAL HYPERKINETIC MOVEMENT DISORDERS

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Introduction: Focal muscular hyperactivity resulting from impaired inhibitory neurotransmission at different CNS levels, including ventral horn and brainstem motor nuclei, is a common symptom of different movement disorders like dystonias, post stroke spasticity, and spinal cord injuries. Presently, rodent in vivo studies of muscular hyperactivity disorders are based on spinal lesions or stroke models, which cannot target individual muscles. In this study we employed low-dose intramuscular (i.m.) tetanus toxin (TeNT) to model focal muscular hyperactivity.

Materials and methods: Local muscle spasm was induced by low dose i.m. TeNT into rat gastrocnemius (1-1.5 ng). Pharmacological response to botulinum toxin A (BoNT/A) (5 U/kg) and intraperitoneal R(+) baclofen (3 mg/kg) was assessed by different motor parameters and tests in conscious animals: dorsiflexion resistance, tibioplantar angle, swimming velocity, rota-rod, and beam walking.

Antinociceptive effect of botulinum toxin type A on persistent bilateral allodynia induced by intramuscular carrageenan in rats

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Introduction

"Mirror pain" or "mirror-image pain" (MP) is a phenomenon in which unilateral injury results in bilateral pain sensation. Although described in many case reports in humans, the precise frequency of the disorder was never investigated. Treatment varies from case to case and there is no consent. Based on experimental research, it is speculated that mechanisms of contralateral spread of pain involve yet unidentified spinal neural pathways (reviewed by Koltzenburg et al. 1999; Shenker et al. 2003; Janalcek 2011) with critical contribution of deregulated supraspinal descending pathways (Radhakrishnan and Sluka 2009).

Botulinum toxin type A (BT-A) has long-lasting antinociceptive effect after local application, which is not associated only with site of application, but spreads on distant sites as well: it exerts bilateral antinociceptive effect following unilateral application (Bach-Rojecky and Lacković, 2009; Favre-Guilmard et al., 2009; Bach-Rojecky et al., 2010; Filipović et al. 2012; Lacković et al. 2016). In the present study we characterize the localization of antinociceptive effect of botulinum toxin type A (BT-A) in MP induced by intramuscular λ-carrageenan injection. Additionally, we investigate the role of spinal glial cells in MP and in the effect of BT-A.

Materials & Methods

100 μL 3% carrageenan (dissolved in saline) or saline was injected into right gastrocnemius muscle of male Wistar rats. Animals which developed bilateral mechanical allodynia two weeks following carrageenan injection were divided into following groups: (1) and (2) saline or BT-A (5 U/kg) subcutaneously into the right hind-paw pad (ipsilateral i.pl.), (3) BT-A (5 U/kg) subcutaneously into the left hind-paw pad (contralateral i.pl.), (4) and (5) saline or BT-A (1 U/kg) in the spinal canal (i.t.), (5) and (6) saline or BT-A (1 U/kg) into the cisterna magna (i.c.). Nociceptive measurements were performed using von Frey filaments. Glial activation markers (GFAP for astrocytes and CD11b for microglia) in the lumbar spinal cord (L4) were assessed by immunohistochemistry. Data were analyzed using One way-ANOVA followed by Tukey post hoc test ($p<0.05$).

* GFAP • Glial Fibrillary Acidic Protein; CD11b • Cluster of Differentiation 11B

Results

- Unilateral intramuscular carrageenan injection produced acute unilateral sensitivity to mechanical stimuli that spread contralaterally within 1-2 weeks ($p<0.01$ compared to control)
- Ipsilateral i.pl. BT-A decreased mechanical hypersensitivity on the ipsilateral, but also on the contralateral side ($p<0.01$) 5 days following application
- Bilateral reduction of mechanical hypersensitivity to von Frey filaments ($p<0.01$) was also observed 1 day following BT-A (1 U/kg) i.t. administration
- Contralateral i.pl. and i.c. BT-A injection had no effect on pain on either side

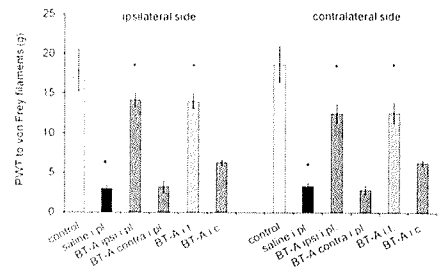

Fig. 1 Peripheral and spinal, but not supraspinal, BT-A reduces MP induced by i.m. carrageenan injection

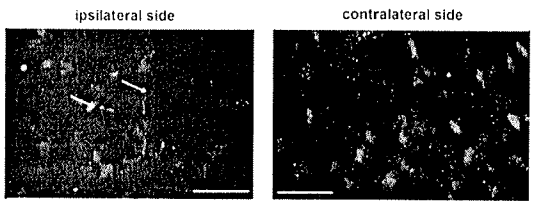

Fig. 2 Cleaved SNAP-25 in spinal dorsal horn, as an evidence of spinal site of BT-A action, present only at ipsilateral side

- The bilateral BT-A's antinociceptive effect, as well as MP induced by i.m. carrageenan does not involve glial cell activation

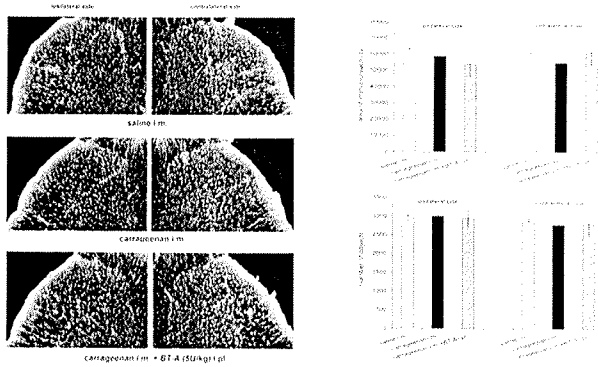

Fig. 3 Qualitative and quantitative analysis of GFAP expression in L4 lumbar spinal cord

Selected additional data from our most recent study (Drinovac et al., 2016)

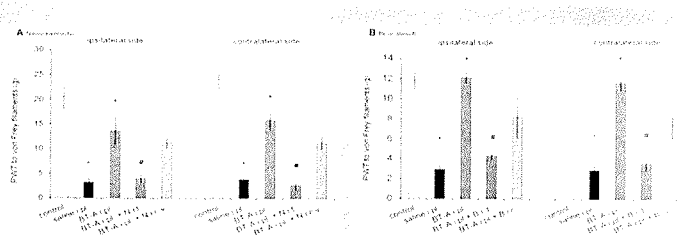

Fig. 4 Bilateral antinociceptive effect of BT-A in i.m. carrageenan induced MP involves interaction with spinal opioid and GABAergic system

Conclusion

BT-A's bilateral antinociceptive effect can be achieved after peripheral ipsilateral and intrathecal, but not intracisternal application. This might indicate that its effects on MP, as well as the interaction with opioid and GABAergic systems, occur at segmental level of the spinal cord and does not involve spinal glia. BT-A is the only substance known so far with bilateral effect in different types of experimental MP after unilateral application. Possible benefit in cases of human bilateral pain might be important to investigate.

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