## BLOCKADE OF GLUTAMATE MGLU5 RECEPTORS PREVENTS THE MORPHOLOGICAL CHANGES IN DORSAL HORN LAMINA II IN A MODEL OF NEUROPHATIC PAIN

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INTRODUCTION

Neurophatic pain is characterized by hyperalgesia and allodynia to mechanical and thermal stimuli, which cause morphological changes in spinal dorsal horn. Peripheral nerve injury results in the vocation of synaptic sites within the substantia gelatinosa of superficial dorsal horn as a consequence of transganglionic degeneration (Whiteside GT and Munglani R, 2001; Maione et al. 2002). Atrophy of nonmyelinated C fibers has been implicated in this synaptic loss. Moreover, neurophatic pain elicits long-lasting sprouting of A-fibers into lamina II, an area in which they do not normally terminate, and inappropriate synaptic formation by the sprouting A-fibers. This degenerative and regenerative changes result in a structural reorganization of highly ordered laminar synaptic termination field in the dorsal horn of the spinal cord, which may modify sensory input to the CNS. The best known candidate to be potentially able to induce central sensitisation is glutamate. Spinal ionotropic and metabotropic glutamate receptors clearly contribute to this and changes in downstream post-receptorial system are required. This study combines behavioural and morphological approaches to asses the occurrence of apoptosis, amyelinated and myelinated fibers changes at the lumbar (L4-L5) spinal cord of rats by 1, 3, 7 and 14 days sciatic nerve chronic constrictive injury (CCI). Moreover, the effect of MPEP, a selective mGlu5 receptors antagonist, was evaluated on behavioural and morphological changes induced by CCI.

METHODS

The animals were perfused through the left ventricle with 100 ml saline followed by 300 ml of Zamboni's solution. Segments $L_4$-$L_5$ of the spinal cord were removed and kept in the same fixative overnight and then cryoprotected in 30% sucrose in 0.1 M PBS. Frozen sections were cut at 50 ìm with a freezing slide microtome. Sections were then incubated in a combination of primary antibodies overnight to rabbit anti-protein gene product (PGP 9.5) a marker of neurones, rabbit anti-glial fibrillary acidic protein (GFAP) a marker of glia, rabbit anti-substance P (SP) for C-fibers, and mouse anti-myelin basic protein (MBP) for A-fibers. Secondary antibodies specific to the IgG species used as a primary antibody and labelled with cyanine dye fluorophores 3.18 and 5.18. After immunohistochemical processing, sections were adhered to coverslips with agar, dehydrated via an alcohol series, cleared with methyl salicylate, and mounted in DPX.
Nonadjacent section from the L4-L5 lumbar spinal cord (n=5-8) were randomly selected using a Zeiss LSM 410 Invert and digitized. Quantification of numbers of PGP 9.5-ir neurons and GFAP-ir astrocytes and MBP-ir A-fibers in the superficial dorsal horn, was performed with Neurolucida Software using a 20X plan apochromat objective by an observer blind to the treatment. To quntifity the density of SP labelling section were captured with a 4X objective. Then, the areas of the section to be analyzed were outlined using NIH Image Software, and the integrade optical density (IOD) of staining in each outlined area was measured.

RESULTS

Three days post-surgery the rats showed a significant ($p<0.05$) negative mean difference score for paw withdrawal latency to radiant heat and mechanical stimulation. The increased sensitivity on the neurophatic side was interpreted as thermal and mechanical hyperalgesia.
Immunohistochemical analysis, by confocal microscopy, showed a decrease (52±6%) in PGP 9.5-ir positive neurones in the laminae I-III ipsilateral to CCI. Changes in the size and shape of the astrocytes cell body (reactive gliosis) in lamina II ipsi\ lateral to CCI was observed. Apoptosis occurred as confirmed by TUNEL-positive nuclei in this territory. Moreover, a decrease of amyelinated C-fibers (-37±7%), followed by sprouting of myelinated A-fibers ipsilateral to injury, has been observed as well.
Groups of rats (n=5) were also treated chronically with MPEP (2 mg/Kg i.p. twice, daily), a selective mGlu5 receptors antagonist. This treatment (commenced the same day of the CCI) resulted to be effective in preventing the appearance of thermal hyperalgesia as measured at all times with plantar test but resulted to be transiently (3 days post-surgery) effective in preventing mechanical hyperalgesia as this reappeared by 7 days

CCI. Morphological analysis showed that such a neurons reduction was abolished in rats treated with the glutamate mGlu5 receptor antagonist MPEP by 3 days post-CCI, but was unable to prevent the neuronal loss 7 days post-CCI. Reactive gliosis in lamina II was reduce by 35 ±6%. Moreover, the blockade of the mGlu5 receptors led to a substantial protection on amyelinated C-fibers and a reduction of sprouting of myelinated A-fibers.

CONCLUSION

Glutamate mGlu5 receptors play a critical role in transient spinal cytoarchitecture modification involved in the genesis of hyperalgesia/allodynia.

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