INTRODUCTION

Glutamate synthesis in neurons occurs by two enzymes, aspartate aminotransferase (AST) and glutaminase (GLS). Our previous studies examined temporal alterations in AST and GLS expression in rat dorsal root ganglion (DRG) neurons during a somatic inflammation model, adjuvant-induced arthritis (AIA; Miller et al., 2012). We noted a temporal biphasic expression of GLS and AST in DRG neurons. In preliminary experiments using a visceral inflammatory model, trinitrobenzene sulphonate (TNBS)-induced colitis, we observed both acute and chronic changes in AST and GLS in DRG neurons. In the current study, our aim was to determine the temporal expression of AST and GLS in rat DRG neurons during TNBS-induced colitis.

METHODS

• Colitis was produced via intracolonic infusion of trinitrobenzene sulphonate (TNBS; 50 mg/kg; 0.5 ml).
• On days 1, 2, 4, 8, 16, 30 of colitis, sacral 1 (S1) DRG were fixed (Hoffman et al., 2010), isolated, and processed for GLS- and AST-immunoreactivity (ir). DAPI was used to stain for nuclei.
• Image analysis of DRG neurons was performed with Image J. Each DRG neuron was evaluated to determine a mean gray intensity (MGI). Total MGI per DRG from individual neuron MGI’s was determined for each rat.

RESULTS

Colitis causes increased GLS- and AST-ir in DRG neurons

Fig 1. GLS-ir occurs in all DRG neurons (A-arrows). Compared to controls, rats with colitis (day 2) had an increase in GLS-ir in S1 DRG neurons (B-arrows). A decrease in GLS-ir occurred at day 4 (C, arrows), followed by a second increase at day 8 (D, arrows). GLS-ir remained elevated at days 16 and 30. Similar results occur in AST-ir in S1 DRG neurons during colitis.

Microscopy image intensities from individual neurons (A) can be plotted (B) and these data can be quantified.

Neuron profiles containing a nucleus are outlined as cells for analysis (C,D). DAPI nuclei are outlined (E). Total area and cytoplasmic MGI per neuron are determined.

Quantitative analysis of GLS- and AST-ir during colitis shows an elevation at early time points (Days 1-2; 25-60%), near normal levels at Day 4, and an increase at Days 8-16 (30-40%). GLS- and AST-ir remain elevated at Day 30 (5-15%). *** p<0.001, ** p<0.01, * p<0.05

REFERENCES

• Hoffman et al., Fixative composition alters distributions of immunoreactivity for glutaminase and two markers of nociceptive neurons, Nav1.8 and TRPV1, in the rat dorsal root ganglion. J. Histochem. Cytochem. 5:329-44, 2010.
• Miller K.E., Glutaminase immunoreactivity and enzyme activity is increased in the rat dorsal root ganglion following inflammation. Pain Research & Treatment 2012:414697, 2012.

SUMMARY

Elevated AST and GLS levels in DRG neuronal perikarya leads to increased glutamate production in peripheral and central terminals. The hypersensitivity observed in colitis may be due to altered glutamate synthesis and release. Interventional therapies for diminishing altered glutamate synthesis may hold promise for pain relief in visceral inflammation.

REFERENCES

• Hoffman et al., Fixative composition alters distributions of immunoreactivity for glutaminase and two markers of nociceptive neurons, Nav1.8 and TRPV1, in the rat dorsal root ganglion. J. Histochem. Cytochem. 5:329-44, 2010.
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