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Propentofylline-Induced Astrocyte Modulation Leads to Alterations in Glial Glutamate Promoter Activation Following Spinal Nerve Transection

Vivianne L. Tawfik¹, Melissa R. Regan², Christine Haenggeli², Michael L. LaCroix-Fralish¹, Nancy Nutile-McMenemy⁴, Natalie Perez², Jeffrey D. Rothstein^{2,3}, and Joyce A. DeLeo^{1,4}

¹Department of Pharmacology, Dartmouth Medical School, Hanover, NH 03755

²Department of Neurology, Johns Hopkins University, Baltimore, MD 21287

³Department of Neuroscience, Johns Hopkins University, Baltimore, MD 21287

⁴Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, Lebanon, NH 03756

Abstract

We have previously shown that the atypical methylxanthine, propentofylline, reduces mechanical allodynia after peripheral nerve transection in a rodent model of neuropathy. In the present study, we sought to determine whether propentofylline-induced glial modulation alters spinal glutamate transporters, GLT-1 and GLAST *in vivo*, which may contribute to reduced behavioral hypersensitivity after nerve injury. In order to specifically examine the expression of the spinal glutamate transporters, a novel line of double transgenic GLT-1-eGFP/GLAST-DsRed promoter mice was used. Adult mice received propentofylline (10 mg/kg) or saline via intraperitoneal injection starting 1-hour prior to L5-spinal nerve transection and then daily for 12 days. Mice receiving saline exhibited punctate expression of both eGFP (GLT-1 promoter activation) and DsRed (GLAST promoter activation) in the dorsal horn of the spinal cord, which was decreased ipsilateral to nerve injury on day 12. Propentofylline administration reinstated promoter activation on the injured side as evidenced by an equal number of eGFP (GLT-1) and DsRed (GLAST) puncta in both dorsal horns. As demonstrated in previous studies, propentofylline induced a concomitant reversal of L5 spinal nerve transection-induced expression of Glial Fibrillary Acidic Protein (GFAP). The ability of propentofylline to alter glial glutamate transporters highlights the importance of controlling aberrant glial activation in neuropathic pain and suggests one possible mechanism for the anti-allodynic action of this drug.

Keywords

Spinal glia; Neuropathic pain; Neuroimmune; Peripheral nerve injury; Mice

Neuropathic pain is a debilitating condition that affects millions of individuals worldwide. Unfortunately, treatment options for these patients are limited as opioids and other available pharmacotherapies are not able to adequately control associated spontaneous pain, allodynia and hyperalgesia long-term in many patients. In an effort to uncover mechanisms that could

Corresponding author: Joyce A. DeLeo, PhD, 1 Medical Center Drive, HB 7125, Lebanon, NH 03756, (603) 650-6204 phone, (603) 650-4928 fax, Joyce.A.DeLeo@Dartmouth.edu.

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lead to novel drug targets, our laboratory has focused on the role of spinal neuroimmune activation in nerve injury-induced behavioral sensitization. As a result, we have demonstrated that peripheral nerve damage induces activation of spinal astrocytes and microglia by enhanced expression of both surface antigens and functional proteins and increased cytokine expression that correlates with behavioral hypersensitivity (DeLeo et al., 1997; Colburn et al., 1999).

The contribution of activated glia to nerve injury-induced mechanical allodynia has been further elucidated using propentofylline, an atypical methylxanthine previously shown to attenuate astrocytic activation in a rodent model of ischemia (DeLeo et al., 1987). Systemic or intrathecal propentofylline attenuated mechanical allodynia induced by L5 spinal nerve transection and this effect correlated temporally with a reduction in GFAP and CR3/CD11b expression (Sweitzer et al., 2001). Although the specific mechanism of propentofylline remains unknown, several actions have been proposed. Propentofylline has been shown to inhibit adenosine transport and the cyclic-adenosine-5',3'-monophosphate (cAMP)-specific phosphodiesterase (PDE IV) leading to induction of cAMP (Nagata et al., 1985; Parkinson and Fredholm, 1991; Meskini et al., 1994). Strengthening of cAMP-dependent signaling decreases microglial proliferation and activation in culture (Si et al., 1996), providing a possible mechanism of glial modulation via propentofylline.

Glia contribute to synaptic homeostasis by releasing neurotrophic factors and preventing glutamate excitotoxicity by promoting Na⁺-dependent glutamate uptake (Liberto et al., 2004). To date, five glutamate transporters have been identified (Danbolt, 2001). EAAT1/GLAST and EAAT2/GLT-1 are thought to be primarily localized to astrocytes although they have recently been demonstrated to be expressed on microglia (Lopez-Redondo et al., 2000) and neurons (Chen et al., 2004). Activated astrocytes may lose their homeostatic functions upon exposure to stressors and increase the expression of cytokines, nitric oxide and prostaglandins as an injury response (Watkins et al., 2001). Prior studies have determined that post-chronic constriction injury (Sung et al., 2003) or facial nerve axotomy (Lopez-Redondo et al., 2000) glial glutamate transporters are decreased, corresponding temporally to our observation of enhanced astrocytic activation (Sweitzer et al., 2001; Tanga et al., 2004).

In the current study, we sought to determine how propentofylline-induced suppression of glial activation might contribute mechanistically to reduced behavioral hypersensitivity after nerve injury. We selected the L5 spinal nerve transection model of neuropathic pain because of the robust and reproducible mechanical allodynia produced (Sweitzer et al., 2001; Tawfik et al., 2005). We took advantage of a novel double transgenic reporter mouse line expressing eGFP at GLT-1 promoter activation and DsRed at GLAST promoter activation (Regan et al., 2007) to determine the effect of propentofylline on these specific transporters. We show that spinal GLT-1 and GLAST are altered following L5 nerve transection and that propentofylline treatment is capable of reversing these changes in a manner that is temporally correlated with reversal of mechanical allodynia.

Methods

Animals

Promoter reporter transgenic mice on a C57Bl/6 background were created according to Regan et al. (2007). Briefly, to determine where the GLAST promoter is activated, transgenic mice were created using a mouse bacterial artificial chromosome (BAC) containing the GLAST gene plus 18 kb of DNA upstream of the first exon and 60 kb downstream of the last exon. DsRed cDNA was inserted into the first exon to allow expression of DsRed instead of GLAST when the promoter is active. Similarly, to investigate activation of the GLT-1 promoter, transgenic mice were created using a mouse BAC containing the GLT-1 gene plus 45 kb upstream and 24 kb downstream with cDNA for eGFP inserted into the start codon. These two

lines of transgenic mice were crossed, and the resultant double transgenic GLT-1-eGFP/GLAST-DsRed mice were used in this study at 8–10 weeks of age. Efforts were made to limit animal distress and to use the minimum number of animals necessary to achieve statistical significance, in accord with guidelines set forth by the International Association for the Study of Pain (Covino, 1980). The Institutional Animal Care and Use Committee at Dartmouth College approved all procedures in this study.

L5 spinal nerve transection surgery

Unilateral mononeuropathy was produced according to the method described by Colburn et al. (1999). Briefly, mice were anesthetized by inhalational halothane in an O₂ carrier (induction, 4%; maintenance, 2%). A small incision to the skin overlying L5–S1 was made, followed by retraction of the paravertebral musculature from the superior articular and transverse processes. The L6 transverse process was partially removed, exposing the L4 and L5 spinal nerves. The L5 spinal nerve was identified, separated, lifted, and transected, followed by removal of a 3-mm distal segment of nerve to prevent reconnection. The wound was irrigated with saline and closed in two layers with 3-0 polyester suture (fascial plane) and surgical skin staples. Sham surgery was identical except that the nerve was exposed and lifted but not transected.

Mechanical allodynia

Mechanical sensitivity to non-noxious stimuli was measured by applying von Frey filaments (Stoelting, Wood Dale, IL) to the plantar surface of the ipsilateral hind paw ($n = 5$ –8/group). Filament weights used were 0.008 g and 0.02 g. Each round of testing consisted of 3 sets of 10 stimulations, with sets separated by 10 minutes from the previous (to avoid sensitization), for a total of 30 stimulations with each filament. The number of paw withdrawals observed is expressed out of a maximum of 30 possible withdrawals.

Immunohistochemistry

Mice were anesthetized with an overdose of 4% chloral hydrate then transcardially perfused with 0.1 M PBS followed by 4% PFA. Lumbar spinal cord sections were identified, removed and post-fixed in 4% PFA for 2 hours. After one week in 30% sucrose to prevent freeze fracture, spinal cord segments were frozen in dry ice and mounted in Optimal Cutting Temperature (O.C.T., Sakura FineTek, Torrance, CA) for cryostat sectioning. Twenty micron serial sections were processed for GFAP immunoreactivity. Lumbar spinal cord sections were identified, isolated, and processed as described previously (Colburn et al., 1997). Sections were blocked with 5% normal goat serum (NGS, Sigma, St. Louis, MO)/0.01 % Triton-X 100/PBS for 1 h at room temperature (RT). Spinal sections were incubated with mouse anti-GFAP G-A-5 (astrocyte marker, 1:400, Sigma) in 3% NGS/0.01 % Triton-X 100/PBS over night at 4°C, washed, and incubated in goat anti-mouse Alexa Fluor™-405 (1:250, Molecular Probes, Eugene, OR) secondary antibody in 3% FBS/0.01% Triton-X 100/TBS for 1 h at RT. To control for nonspecific staining, control sections were incubated in the absence of primary antibodies. The resultant sections were mounted with Vectashield (Vector Laboratories, Burlingame, CA). Some sections were not processed for GFAP but simply mounted with DAPI, a nuclear stain which also fluoresces in the 405 nm range, in order to determine the cellular localization of GLT-1 and GLAST in the sections. The sections were examined with an Olympus fluorescence microscope and out of three sections, a representative spinal cord section for each mouse based on quantitative assessment (as described below) was captured with a Q-Fire cooled camera. For both eGFP and DsRed, images were taken with a 397 ms exposure. For GFAP/Alexa 405, images were taken with a 131 ms exposure.

The line of double transgenic mice used in these experiments exhibits a distinct pattern of GLT-1 and GLAST promoter activation in the spinal cord composed of both punctate, perinuclear expression as well as diffuse, cytoplasmic staining. We quantified the relative

number of eGFP (GLT-1) or DsRed (GLAST) puncta in each spinal cord, in the entire region of the dorsal horn of the spinal cord, using Adobe Photoshop 7.0 (Adobe Systems, San Jose, CA). Specifically, images taken with the 488 nm laser, showing eGFP, were altered by decreasing the brightness to -52 units and increasing the contrast to +71 units. With this change (carried out identically on all images used) bright eGFP positive puncta were easily identifiable and counted by an observer blind to the treatment groups. Images taken using the 555 nm laser, showing DsRed staining, were altered by decreasing the brightness to -12 units and increasing the contrast to +65 units. Again, all images were processed identically and DsRed positive puncta were counted. Results are expressed as the relative number of GLT-1 or GLAST positive puncta in the ipsilateral (injured) dorsal horn vs. the contralateral (non-injured) dorsal horn.

***In Vivo* Experimental Design**

For the transgenic eGFP-GLT-1/DsRed-GLAST mice, 10 mg/kg propentofylline (Sigma, Inc. St. Louis, MO) in sterile, non-pyrogenic saline (Abbott Laboratories, North Chicago, IL), or saline vehicle alone, was administered intraperitoneally (i.p., $n = 5-8$ /group). This dose was selected based on prior studies from our laboratory demonstrating a clear suppression of mechanical allodynia in rats at 10 mg/kg (Sweitzer et al., 2001). Treatment began one hour prior to L5 spinal nerve transection and continued daily in the evening (between 5:00 and 7:00 pm) until day 12 post-transection. Mice received either an L5 spinal nerve transection or sham surgery on day 0. The development of mechanical allodynia was monitored by a blinded observer on days 1, 3, 5, 7, 9 and 12 as described above, in the morning at approximately 15 hours post-propentofylline or saline injection. Mice were transcardially perfused on day 12 post-transection; spinal cords were removed and further processed for immunohistochemistry as described above.

Data analysis

Values are expressed as means $\pm$ SEM. For behavioral data, comparisons between groups were performed using two-way analysis of variance (ANOVA), followed by Bonferroni post-tests, with treatment group and length of exposure (day) as factors. GLT-1 and GLAST cell count data were analyzed using a one-way ANOVA followed by Tukey multiple comparisons post-test. In all cases, $P < 0.05$ was considered significant.

Results

Propentofylline decreases allodynia in mice after L5 spinal nerve transection

After L5 spinal nerve transection, mice developed mechanical allodynia to both 0.008 g (Figure 1A: *** $P < 0.001$; Sham/sal vs. L5/sal) and 0.02 g (Figure 1B: ** $P < 0.01$, *** $P < 0.001$; Sham/sal vs. L5/sal) von Frey filaments. Daily treatment with propentofylline, initiated one hour prior to transection, robustly inhibited the development of mechanical allodynia in mice starting at day 1 for the 0.02 g filament ($^aP < 0.01$, # $P < 0.001$; L5/sal vs. L5/PPF) and day 3 for the 0.008 g filament ($^+P < 0.05$, # $P < 0.001$; L5/sal vs. L5/PPF). The L5/PPF group remained similar to the sham groups at every time point for both filaments ($P > 0.05$, ns; Sham/sal vs. L5/PPF).

Transgenic reporter mice allow for specific quantitation of GLT-1 and GLAST alterations

As shown at high power, the transgenic mice demonstrate two types of staining in the dorsal horn of the spinal cord (Figure 2). For both reporters (GLT-1 in green; GLAST in red) diffuse, cytoplasmic (Figure 2, arrowhead) and punctate, perinuclear (Figure 2, arrow) expression is seen that overlaps with GFAP (shown in blue, top row) in some, but not all cases. The perinuclear location of the puncta is highlighted by co-labeling with the nuclear stain, DAPI, shown in Figure 2, bottom row. As shown previously in rats, L5 spinal nerve transection leads

to an enhancement of GFAP, most notably on the side ipsilateral to the injury (Figure 3, L5/saline). In addition, we demonstrate that propentofylline treatment suppressed injury-induced GFAP expression in the dorsal horn upper laminae (Figure 3, L5/PPF).

Each mouse exhibited a unique pattern of eGFP-GLT-1 expression in the dorsal horn of the spinal cord (Figure 4). While there was significant animal-to-animal variation in the number of eGFP positive puncta per mouse, there was consistency across the dorsal horns for a given animal, making it possible to compare injured vs. non-injured side effects. Figure 4 shows representative images of ipsilateral and contralateral lumbar dorsal horn for all groups. Both the Sham/saline and the Sham/PPF groups displayed equivalent numbers of eGFP-GLT-1 puncta in both spinal cord dorsal horns, indicating that propentofylline treatment did not lead to further increases in GLT-1 above normal. In contrast, L5 spinal nerve transection led to a decrease in the number of eGFP-GLT-1 puncta in the ipsilateral dorsal horn (Figure 4: L5/saline). Treatment of injured mice with propentofylline restored levels of eGFP-GLT-1 to that of the contralateral side (L5/PPF). Quantitation of immunofluorescent puncta demonstrated that L5 spinal nerve transection led to a significant decrease in eGFP-GLT-1 positive cells compared to the Sham/saline group (Figure 6A, $**P<0.01$). In addition, as shown in the photomicrographs, daily propentofylline increased the number of eGFP-GLT-1 positive cells on the injured side and restored the ipsilateral/contralateral ratio to 1 ($**P<0.01$; L5/sal vs. L5/PPF).

Regan et al. (2007) have discussed previously the contribution of the GLAST transporter to glutamate uptake in the spinal cord. They determined that this transporter is expressed in a separate population of cells than is GLT-1 in the spinal cord (see Figure 2) and at a much lower level than GLT-1. This is consistent with what we observed, where very few DsRed-GLAST puncta are observed in the lumbar dorsal horns, and cytoplasmic-type expression of the transgene is considerably lower than that of eGFP-GLT-1 (Figure 5). GLAST expression was noted to be equivalent in Sham/saline and Sham/PPF animals suggesting a lack of effect of PPF alone on transgene expression. These representative images further demonstrate that the absolute number of DsRed-GLAST puncta can vary significantly between animals, but that the ipsilateral vs. contralateral ratio remains close to 1 for a given mouse. As seen with GLT-1, L5 spinal nerve transection led to a decrease in DsRed-GLAST puncta on the ipsilateral side and propentofylline treatment increased levels back to that of the contralateral side. When quantified, a significant effect of propentofylline on the ipsilateral vs. contralateral ratio of GLAST puncta was observed (Figure 6B, $*P<0.05$; L5/sal vs. L5/PPF).

Discussion

We demonstrate that propentofylline (PPF), a methylxanthine derivative that exhibits anti-allodynic properties in a neuropathic pain rodent model, inhibits astrocytic activation and modulates spinal astrocytic promoter activation for glutamate transporters, GLT-1 and GLAST *in vivo*. Using a newly engineered line of transgenic reporter mice, we specifically observed glutamate transporter alterations in the spinal cord dorsal horn and extended our previous findings with propentofylline to mice. Of note, previous work from our laboratories indicates that in primary astrocyte cultures PPF induces a similar suppression of the activated astrocytic phenotype along with enhancement of GLT-1 and GLAST protein (Tawfik et al., 2006) and that there is a close correlation between GLT-1 and GLAST promoter activation and transporter expression and function in spinal cord (Regan et al., 2007). Taken together, these findings suggest that glial activation and glutamate clearance capabilities are inextricably linked and may be an important target for future pain therapeutics.

Glutamate is the primary excitatory neurotransmitter in the CNS and, as such, participates in the majority of crucial brain physiological functions including synaptic transmission and CNS

development (Danbolt, 2001). In order to maintain efficient signaling via glutamate receptors and prevent excitotoxicity, the extracellular concentration of glutamate is exquisitely regulated by a series of sodium-dependent glutamate transporters. Five subtypes of excitatory amino acid transporters have been cloned (Kanai and Hediger, 1992; Pines et al., 1992; Storck et al., 1992; Fairman et al., 1995; Arriza et al., 1997). Antisense and genetic knockout studies have identified GLT-1 as the most crucial in maintenance of low extrasynaptic glutamate levels (Rothstein et al., 1996; Tanaka et al., 1997) while GLAST and possibly EAAC1 play secondary roles.

Excessive accumulation of glutamate can lead to synaptic dysregulation. For example, it has previously been demonstrated that following nerve injury, there is excessive release of excitatory amino acids at the level of the spinal cord. In addition, N-methyl-D-aspartate (NMDA) receptor antagonists, such as MK-801, are capable of reversing injury-induced pain behaviors (Mao et al., 1992; Garrison et al., 1993). It follows that impaired uptake of synaptic glutamate via a decrease in astrocytic glutamate transporters may be, in part, responsible for neuropathic pain-related central sensitization.

Previously, Sung et al. (2003) reported that after chronic constriction injury (CCI) of the sciatic nerve, there was a biphasic alteration in GLT-1 and GLAST protein levels; at days 1 and 4 both transporter proteins were increased, and at days 7 and 14 both were decreased. Another recent study demonstrated that following facial nerve axotomy, GLT-1 protein is decreased ipsilateral to the injury on day 3 (Lopez-Redondo et al., 2000). In the current study, we observed a decrease in both GLT-1 and GLAST promoter activation at day 12, which is consistent with these findings. However, the magnitude of the change was approximately 35% for both GLT-1 and GLAST of the sham surgery control group in our study whereas Sung et al. (2003) report decreases of 48% and 41%, respectively, at day 14. The sciatic nerve receives inputs from L4–L6 and therefore CCI affects a wider range of lumbar spinal cord levels. This may result in a greater neurochemical and metabolic response than does a more proximal L5 spinal nerve transection. In addition, CCI has a marked local inflammatory component (Clatworthy et al., 1995) not present in the spinal nerve transection model which suggests that spinal cord responses may differ. Importantly, the spinal cord has several-fold less GLT-1 than cortex and therefore, it may be more sensitive to perturbations in transporter levels (Regan et al., 2007). This suggests that even a subtle decrease in transporter levels may dysregulate glutamate uptake.

In the current study, we made use of double transgenic reporter mice which allowed us to specifically determine alterations in GLT-1 and GLAST promoter activation levels. An important limitation of our study is that we did not determine transporter function directly; however, previous work in this mouse model has demonstrated that transporter protein and uptake levels correlate well with promoter expression (Regan et al 2007). With this tool, we determined that spinal nerve transection leads to a significant decrease in GLT-1 puncta ipsilateral to the nerve injury. The exact physiological difference between the observed puncta and diffuse expression of GLT-1 and GLAST is not clear. One can speculate that given the perinuclear location of the puncta, these represent sites of newly formed transporter, while the cytoplasmic expression is suggestive of active, membrane localized proteins. It would therefore be interesting to determine, at a later time point, whether the diffuse cytoplasmic staining after injury persists, or whether protein turnover would eventually lead to a reduction in all GLT-1 expression. Propentofylline, in contrast, enhanced GLT-1 and GLAST puncta ipsilateral to the nerve injury. In previous *in vitro* work (Tawfik et al., 2006), we showed that propentofylline enhanced mRNA for GLT-1, which is consistent with our current findings of increased promoter activation.

Importantly, the ultimate decrease in glutamate transporters, regardless of the cause, has been shown to have functional significance in the spinal cord. Recently, it was observed that glutamate uptake was reduced by 72% in the ipsilateral dorsal horn, compared to sham, 4–6 weeks following spinal nerve ligation (SNL) (Binns et al., 2005). In addition, Liaw et al. (2005) demonstrated that inhibition of glutamate transport with DL-threo-β-benzyloxyaspartate (DL-TBOA), produced spontaneous nociceptive behaviors as well as thermal hyperalgesia and mechanical allodynia in rats. Our data indicate that GLT-1 promoter activation is enhanced with PPF treatment. This transporter is responsible for over 90% of synaptic glutamate clearance (Tanaka et al., 1997) giving functional relevance to our observations. Together, these data suggest that the suppression of glial glutamate transporter protein and/or function may be a mechanism common to sensitization, regardless of the model used, as L5 spinal nerve transection or ligation, chronic constriction injury (CCI) and facial nerve axotomy all resulted in ultimate decreases in transporter protein or function.

In this study we demonstrate a possible mechanism for the observed anti-allodynic effect of propentofylline following peripheral nerve injury, namely enhancement of the principally astrocytic glutamate transporter GLT-1. These data may direct future development of drugs that act via modulating glial function. While effective treatment of neuropathic pain remains a challenge for the clinician, the complex nature of this syndrome suggests a multimodal mechanism that may require a multi-target solution. Dissection of key players in central sensitization will result in novel targets for therapy that may provide patients with much needed alternatives and adjunct therapies to relieve their pain.

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Abbreviations

cAMP, cyclic-adenosine-5',3'-monophosphate db-cAMP, dibutyryl-cAMP; GFAP, Glial Fibrillary Acidic Protein; PBS, Phosphate Buffered Saline; PFA, Paraformaldehyde; PPF, Propentofylline.

A) 0.008 g filament

B) 0.02 g filament

Figure 1.

Propentofylline exhibits anti-allodynic properties in a mouse model of neuropathic pain. Sham and L5 spinal nerve transected mice (L5) received daily injections of 10 mg/kg propentofylline (PPF) or saline intraperitoneally, beginning one-hour prior to surgery. Preventative treatment with propentofylline (L5/PPF) resulted in a significant decrease in mechanical allodynia to both 0.008 g (A) and 0.02 g (B) von Frey filaments compared with L5 spinal nerve transected, saline (L5/sal) controls ($^{\#}P < 0.001$). Mechanical allodynia is reported as the average number of paw withdrawals out of $30 \pm \text{S.E.M.}$ ($n = 5-8/\text{treatment}$). Day 0 represents pre-injury responses. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. Sham/sal; $^aP < 0.05$, $^+P < 0.01$ vs. L5/sal.

Figure 2.

Double transgenic eGFP-GLT-1/DsRed-GLAST reporter mice demonstrate unique transporter expression. Top row: Punctate eGFP-GLT-1 and DsRed-GLAST expression is observed (arrows) as well as diffuse, cytoplasmic expression (arrowheads). GFAP (shown in blue) co-localizes with GLT-1 and GLAST in some, but not all cases, suggesting heterogeneity of astrocytes in the spinal cord. Bottom row: Co-labeling with the nuclear stain, DAPI (shown in blue), demonstrates the perinuclear location of GLT-1 and GLAST puncta (arrows). On treatment day 12, mice were transcardially perfused and cryostat sections were stained with mouse anti-GFAP (1:200) followed by an Alexa-Fluor-405 linked secondary (1:250). Images were taken at a magnification of 40x.

Figure 3.

Propentofylline suppresses L5 spinal nerve transection-induced astrocytic activation in mice. GFAP immunoreactivity in the dorsal horn was notably enhanced ipsilateral to the nerve injury on day 12 (L5/saline). Daily treatment with propentofylline dampened astrocytic activation as evidenced by a decrease in GFAP staining in the ipsilateral dorsal horn of the lumbar spinal cord. On treatment day 12, mice were transcardially perfused and cryostat sections were stained with mouse anti-GFAP (1:200) followed by an Alexa-Fluor-405 linked secondary (1:250). Images were taken at a magnification of 20x. Scale bar, 100 μ m.

Figure 4.

Nerve injury leads to a decrease in the number of eGFP-GLT-1 positive cells in the lumbar dorsal horn of transgenic reporter mice. On day 12 after sham surgery with or without propentofylline treatment (Sham/saline and Sham/PPF) eGFP-GLT-1 expression is evenly distributed in both dorsal horns. L5 spinal nerve transection leads to a suppression of eGFP-GLT-1 puncta on the side ipsilateral to the injury suggesting decreased production of the protein. Daily treatment with propentofylline led to reinstatement of the equal ratio between ipsilateral and contralateral dorsal horns. Images were taken at a magnification of 20x. Scale bar, 100 μ m.

Figure 5.

Nerve injury leads to a decrease in the number of DsRed-GLAST positive cells in the lumbar dorsal horn of transgenic reporter mice. On day 12 after sham surgery with or without propentofylline treatment (Sham/saline and Sham/PPF) DsRed-GLAST expression is evenly distributed in both dorsal horns. L5 spinal nerve transection leads to a suppression of DsRed-GLAST puncta on the side ipsilateral to the injury suggesting decreased production of the protein. Daily treatment with propentofylline led to reinstatement of the equal number of puncta between ipsilateral and contralateral dorsal horns. Images were taken at a magnification of 20x. Scale bar, 100 μ m.

Figure 6.

Propentofylline reverses glutamate transporter reduction induced by L5 spinal nerve transection. A) Quantitation of eGFP-GLT-1 puncta in the spinal cord dorsal horn on day 12 post-L5 spinal transection revealed a significant decrease in the L5/saline group (** $P < 0.01$ vs. Sham/sal) which was reversed with propentofylline administration (** $P < 0.01$ vs. L5/PPF). B) DsRed expression (GLAST promoter activation) decreased in L5/saline group. Propentofylline significantly enhanced the number of DsRed-GLAST puncta ipsilateral to the injury (* $P < 0.05$ vs. L5/sal). Images taken at 20x were manipulated as described in Methods, in order to expose only brightly stained puncta in the dorsal horn. eGFAP-GLT-1 or DsRed-GLAST positive cells were then counted and the ratio between ipsilateral and contralateral expression was calculated. Results are expressed as group mean $\pm$ S.E.M.