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Propentofylline, a Glial Modulating Agent, Exhibits Antiallodynic Properties in a Rat Model of Neuropathic Pain

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ABSTRACT

The present study was undertaken to determine whether propentofylline, a glial modulating agent, could both prevent the induction of mechanical allodynia and attenuate existing mechanical allodynia in a rodent L5 spinal nerve transection model of neuropathic pain. In a preventative paradigm, propentofylline (1 and 10 mg/kg intraperitoneally) was administered systemically daily, beginning 1 day prior to nerve transection. This regimen produced a dose-dependent decrease in mechanical allodynia ($p < 0.01$). In another preventative paradigm, propentofylline (0.1, 1, or 10 μg) was administered daily intrathecally via direct lumbar puncture. Intrathecal administration of propentofylline was more effective than systemic administration at dose dependently reducing mechanical allodynia ($p < 0.01$). The effect of systemic propentofylline on existing allodynia was examined with 0.1-, 1-, and 10-mg/kg intraperitoneal administration initiated on day 4 post L5 spinal nerve transection. Systemic propentofylline was found to be equally effective in the attenuation of existing allodynia ($p < 0.01$) as in the prevention of allodynia in this rodent model of neuropathic pain. Spinal cords (L4–L6 segments) were removed for immunohistochemical analysis on day 10 or 20 post-transection. Microglial and astrocytic activation was decreased by both peripheral and central administration of propentofylline in both preventative and existing allodynia paradigms. This research supports a growing body of literature highlighting the importance of glial activation in the development of persistent neuropathic pain states, and the potential to therapeutically modulate glial activation in the treatment of neuropathic pain.

Chronic neuropathic pain is a physically and emotionally debilitating condition for which there is no adequate treatment. It is a condition that is often refractory to opioids or requires large doses that possess unacceptable side effects. In addition, long-term opioid therapy is limited by the development of tolerance and physical dependence. In an effort to develop novel therapeutic targets for the treatment of neuropathic pain conditions, our laboratory has extensively characterized spinal neuroimmune activation, including glial activation, in several rodent models of neuropathic pain (DeLeo and Colburn, 1996; Colburn et al., 1997; Sweitzer et al., 1999).

Following direct assault on the brain by traumatic injury, ischemic insult, or infection, both microglia and astrocytes undergo a stereotypic response in which they quickly take on activated phenotypes (Raivich et al., 1999). Glial activation is characterized by decreased ramification, hypertrophy, proliferation, and the up-regulation of immunoregulatory molecules. When a comparable direct traumatic injury is applied to the spinal cord or the brain it has been observed that the spinal cord responds with significantly more robust glial reaction than that observed to occur following a traumatic brain injury (Schnell et al., 1999). In addition to direct insult on the central nervous system (CNS), a peripheral transection of the facial nerve has also been found to produce robust glial activation within the brain (Blinzinger and Kreutzberg, 1968). Similarly, robust ipsilateral and mild contralateral astrocytic and microglial activation has been characterized within the spinal cord following several peripheral nerve injuries that result in the development of allodynia, a response to a normally non-noxious stimuli (DeLeo and Colburn, 1996; Colburn et al., 1997, 1999; Coyle, 1998). In contrast, no allodynia and mild spinal glial responses were observed following sham surgeries (Colburn et al., 1997, 1999).

Our laboratory has previously shown that in response to hind paw injection of inflammatory formalin or zymosan, or peripheral nerve transection, both microglia and astrocytes in the L5 spinal cord exhibit activated phenotypes. This activation parallels the development of allodynia. In comparison to the short-term inflammatory models of formalin and zymosan, the spinal nerve transection model produces more robust and persistent glial activation that parallels the development of a persistent mechanical allodynia (Sweitzer et

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ABBREVIATIONS: CNS, central nervous system; i.t., intrathecal(ly); GFAP, glial fibrillary acidic protein; PDE, phosphodiesterase; TNF, tumor necrosis factor; IL, interleukin; ICAM, intercellular adhesion molecule.

al., 1999). In addition to the development of glial activation in a temporal pattern that parallels pain behaviors, other laboratories have shown that spinal administration of a glial metabolic inhibitor, fluorocitrate, attenuates hyperalgesia in both formalin (Watkins et al., 1997) and zymosan (Meller et al., 1994) models of acute inflammatory pain. These data suggest that activated glial cells participate in the initiation and maintenance of acute inflammatory pain states.

In an effort to understand the contribution of glial activation to the development of mechanical allodynia following L5 spinal nerve transection in the rat, we have used the compound propentofylline [3,7-dihydro-3-methyl-1-(5-oxohexyl)-7-propyl-1*H*-purine-2,6-dione]. Propentofylline is a methylxanthine derivative previously found to attenuate astrocytic activation in a rodent ischemia model (DeLeo et al., 1987). In ischemia, propentofylline has been shown to be neuroprotective through a multitude of actions, including inhibition of glutamate release (Andine et al., 1990; Miyashita et al., 1992) and increased nerve growth factor secretion (Shinoda et al., 1990). In vitro studies revealed that propentofylline maintains astrocytic glutamate uptake and inhibits potentially neurotoxic functions adopted by microglia upon pathological activation (Schubert et al., 2000). In addition, systemic application of propentofylline has been found to inhibit lumbar spinal microglial activation following middle cerebral artery occlusion (Wu et al., 1999). Thus, in the current study we determined whether propentofylline produces a decrease in mechanical allodynia in a rodent model of neuropathic pain and whether these antiallodynic properties of the compound are secondary to blockade of glial activation.

Materials and Methods

Animals. Male Holtzman rats (Harlan, Indianapolis, IN) were used. All experimental protocols were approved by the Institutional Animal Care and Use Committee at Dartmouth College. Efforts were made throughout the experiment to minimize animal discomfort and to reduce the number of animals used. All rats (200–250 g at time of nerve transection) were housed in a 12-h light/dark cycle (7 AM lights turned on) with food and water available ad libitum.

L5 Spinal Nerve Transection. Rats were anesthetized with halothane in O_2 carrier (induction 4%, maintenance 2%). A small incision to the skin overlaying L5-S1 was made followed by retraction of the paravertebral musculature from the vertebral transverse processes. The L6 transverse process was partially removed exposing the L4 and L5 spinal nerves. The L5 spinal nerve was identified, lifted slightly, and transected. The wound was irrigated with saline and closed in two layers with 3-0 polyester suture (fascial plane) and surgical skin staples.

Prevention of Peripheral Nerve Injury-Induced Mechanical Allodynia. In a systemic preventative paradigm 1 or 10 mg/kg propentofylline (Aventis, Inc., Frankfurt, Germany) or saline vehicle (n = 8/treatment) was administered by the i.p. route. Treatment was initiated 1 day prior to surgery and continued daily to day 10 post-transection. Mechanical allodynia was tested in the morning at 15 h post-treatment.

In a central preventative paradigm, 0.1 μg (8.16 μM), 1 μg (81.6 μM), or 10 μg (816 μM) of propentofylline in 40 μl of sterile saline (pH 6–7) was injected intrathecally (i.t.) daily via lumbar puncture at the L4/5 level under brief halothane anesthesia (n = 8/treatment). Administration was initiated the afternoon before surgery. Drug administration on the day of surgery preceded the operation by 1 h. Daily administration was in the early evening and continued to day 10 post-transection. Propentofylline treatment preceded mechanical allodynia testing by 15 h.

Attenuation of Established Peripheral Nerve Injury-Induced Mechanical Allodynia. Propentofylline (0.1, 1, and 10 mg/kg) was administered i.p. in an existing allodynia strategy (n = 12/treatment). Daily i.p. drug administration was initiated on day 4 post-transection and continued for the duration of the study. All injections were completed 15 h prior to mechanical allodynia testing.

A crossover study (n = 8/treatment) with central 1 μg of administration of propentofylline was completed. One group of animals was treated with a preventative pain paradigm in which treatment was initiated 1 day prior to L5 spinal nerve transection and continued daily to day 7 post-transection. At day 7 post-transection propentofylline treatment was terminated and the animals were followed out to day 14. A second group of animals, in an existing pain paradigm, began daily i.t. 1-μg propentofylline treatment on day 7 post-transection and continued to day 14.

Mechanical Allodynia. As previously described (Sweitzer et al., 1999), all animals were tested for mechanical allodynia with 2- and 12-g von Frey filaments (Stoelting, Wood Dale, IL) on the ipsilateral hindpaw. Animals were acclimated to the testing procedure. Three baseline measurements were collected before the day of surgery. Rats were subjected to three sets of 10 stimulations with each filament with at least 10 min between each set of stimulations to prevent sensitization. Allodynia was characterized as an intense withdrawal of the paw to this normally non-noxious stimulus. Results are reported as the average number of paw withdrawals out of 30 stimulations with either the 2- or 12-g von Frey filament. Mechanical allodynia was tested on days 1, 3, 5, and 7 for the prevention studies. Mechanical allodynia was tested on days 1, 3, 5, 7, 11, 13, 15, 17, and 20 for the existing study, and days 1, 3, 5, 7, 8, 10, 12, and 14 for the crossover study. Mechanical allodynia testing occurred between 7 AM and 9 AM, 15 h postdrug administration.

Immunohistochemistry. Animals were deeply anesthetized with sodium pentobarbital (65 mg/kg i.p.) and euthanized at day 10 or 20 post-transection by transcardiac perfusion. Lumbar spinal cord sections were harvested and processed as previously described (Colburn et al., 1997). Immunohistochemistry was performed on 20 μm of free-floating L5 spinal cord sections. OX-42 (1:2 working dilution; William F. Hickey, Dartmouth Hitchcock Medical Center, Lebanon, NH) was used to label the expression of CR3/CD11b on activated microglia. Glial fibrillary acidic protein (GFAP) (1:20,000 working dilution; Dako Corp., Carpinteria, CA) was used to label astrocytes. Immunohistochemistry was scored blinded to experimental conditions. At least three spinal sections were used to determine scoring for each animal. Scoring was done according to Colburn et al. (1997): o, normal, unactivated tissue; +, mild activation; ++, moderate activation; and +++, intense activation.

Densitometric Analysis of Immunohistochemistry. All tissues analyzed by densitometry were stained in a single immunohistochemical run. This provided for an invariant standard and ensures that the reported results represent a change in staining in the injured tissue and not in the normal tissue. Each individual section of L5 spinal cord (n = 5–6 sections/animal) was imaged on an Olympus DP10 (Olympus America Inc., Melville, NY) digital camera and the image was imported into NIH Image software. A square box of constant size was used to collect four density measurements in the dorsal horn as well as the ventral horn of both the ipsilateral (injured) and contralateral (uninjured) L5 spinal cord. The results are expressed as the percentage change in injured tissue staining compared with normal/naive tissue:

$$\% \text{ change} = \frac{(\text{density of staining injured tissue} - \text{density of staining normal tissue}) \times 100}{\text{density of staining normal tissue}}$$

Statistical Analysis. Data were analyzed for significance with a one-way ANOVA followed by a post hoc Bonferroni analysis using STATA 5.0 (Stata Corp., College Station, TX). p values less than 0.05 were considered significant.

Results

Systemic Prevention of Mechanical Allodynia. Mechanical allodynia increased in a time-dependent manner following L5 spinal nerve transection. Systemic i.p. administration of propentofylline attenuated mechanical allodynia in a rodent L5 spinal nerve transection model (Fig. 1). An overall (across the entire study period) statistically significant reduction in both 2- ($p < 0.01$) and 12-g ($p < 0.01$) mechanical allodynia was observed with 10 mg/kg propentofylline compared with vehicle-treated animals. An overall statistically significant reduction in 12-g ($p < 0.01$) mechanical allodynia was observed with 1 mg/kg propentofylline compared with vehicle-treated animals. A statistically significant attenuation of 2-g mechanical allodynia ($p < 0.05$) was observed on day 5, 7, and 10 post-transection with 1 mg/kg propentofylline compared with saline vehicle.

Central Prevention of Mechanical Allodynia. Propentofylline, administered intrathecally via lumbar puncture in a preventative paradigm was found to attenuate mechanical allodynia resulting from an L5 spinal nerve transection (Fig. 2). An overall statistically significant dose-dependent attenuation, using both 2- ($p < 0.01$) and 12-g ($p < 0.01$) von Frey filaments, was observed with both 1- and 10-μg propentofylline treatment, but not with 0.1-μg propentofylline treatment. There was no statistically significant difference between 1- and 10-μg treatment. Centrally administered 10-μg propentofylline was significantly ($p < 0.01$) more antiallodynic than 10 mg/kg propentofylline administered systemically (Fig. 3).

Systemic Treatment of Existing Mechanical Allodynia. Systemic propentofylline was able to dose dependently attenuate mechanical allodynia using both 2- and 12-g von Frey filaments (Fig. 4). Both 1 and 10 mg/kg propentofylline produce statistically significant ($p < 0.01$) decreases

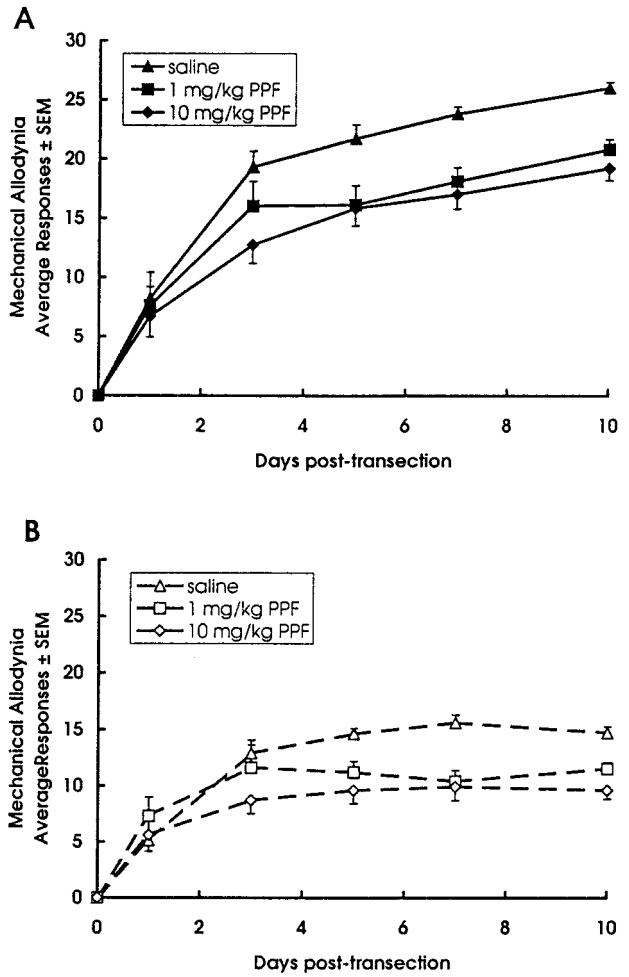

Fig. 1. Systemic prevention of allodynia. Male Holtzman rats received daily intraperitoneal 1 or 10 mg/kg propentofylline (PPF) or saline initiated 1 day prior to L5 spinal nerve transection. Preventative treatment with both 1 and 10 mg/kg propentofylline resulted in an overall statistically significant ($p < 0.05$, $n = 8$/treatment) decrease in both 12- (A) and 2-g (B) von Frey filament-induced mechanical allodynia compared with saline treatment. Mechanical allodynia is reported as the average number of paw withdrawals out of 30 ± S.E.M. Day 0 mechanical allodynia represents baseline pretransection responses.

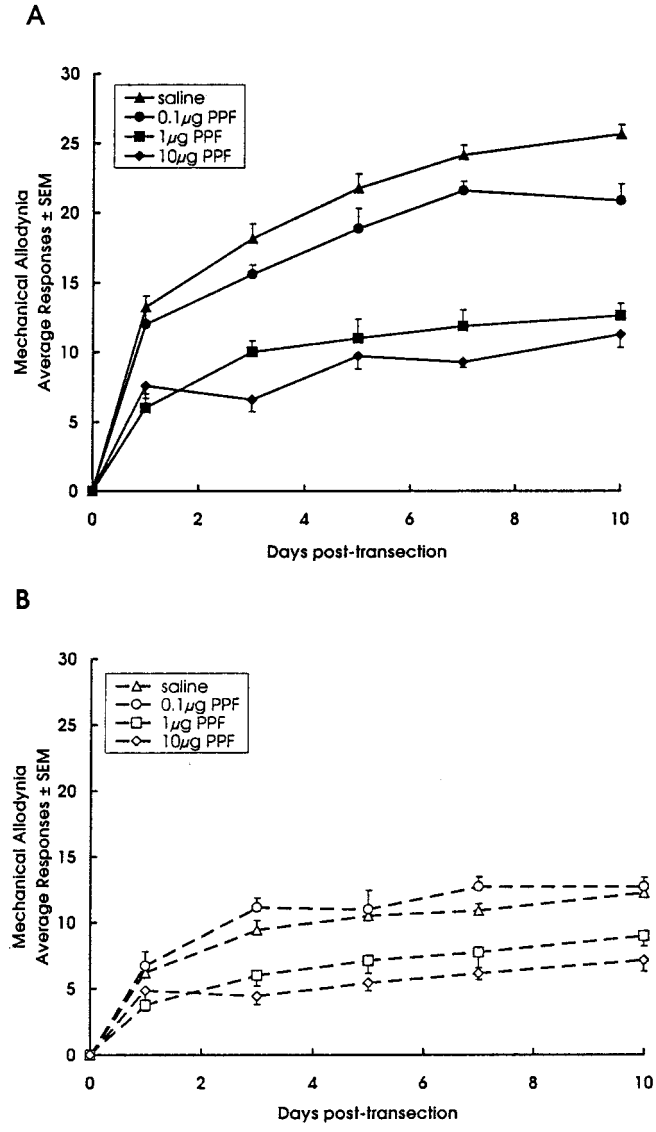

Fig. 2. Central prevention of allodynia. Male Holtzman rats received daily i.t. 0.1, 1, or 10 μg propentofylline (PPF) or saline initiated 1 day prior to L5 spinal nerve transection. Preventative treatment with both 1 and 10 μg of propentofylline produced an overall statistically significant ($p < 0.01$, $n = 8$/treatment) decrease in both 12- (A) and 2-g (B) von Frey filament-induced mechanical allodynia compared with saline or 0.1-μg propentofylline treatment. Mechanical allodynia is reported as the average number of paw withdrawals out of 30 ± S.E.M. Mechanical allodynia on day 0 represents baseline pretransection responses.

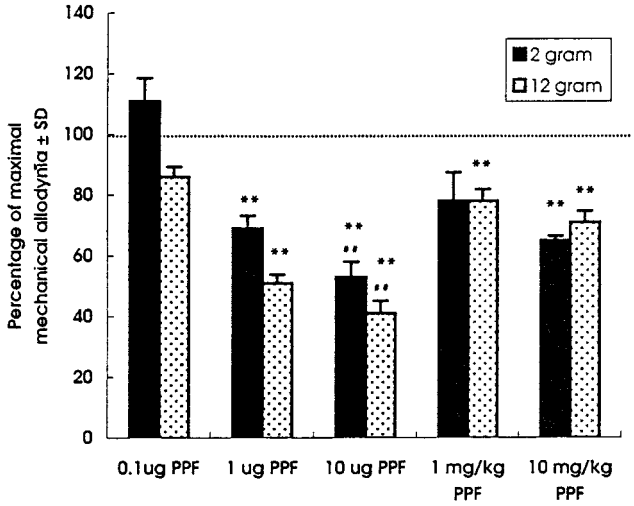

Fig. 3. Comparison of antiallodynic efficacy of systemic versus central administration of propentofylline. Saline-treated animals represent 100% of maximal mechanical allodynia. Both systemic 1 and 10 mg/kg propentofylline and central 1- and 10-μg propentofylline treatment exhibit significantly less 12-g von Frey filament-induced mechanical allodynia compared with saline-treated animals ($^{**}p < 0.01$, $n = 8$/treatment) averaged across the entire time course. Central administration of 10 μg of propentofylline results in significantly less 2- and 12-g von Frey filament-induced mechanical allodynia compared with systemic 10-mg/kg propentofylline treatment ($^{\#\#}p < 0.01$, $n = 8$/treatment).

in mechanical allodynia compared with either vehicle or 0.1 mg/kg propentofylline-treated animals.

Central Treatment of Existing Mechanical Allodynia. In a preventative paradigm, central administration of 1 μg of propentofylline produced a statistically significant attenuation in mechanical allodynia that was sustained for 2 days (day 8 post-transection) following termination of treatment (Fig. 5). Conversely, in the existing allodynia paradigm, a statistically significant reduction in 12-g allodynia was apparent on day 12 and 14 post-transection when propentofylline treatment was initiated on day 7 post-transection (Fig. 5).

Glial Activation in the L5 Spinal Cord following Propentofylline Treatment. Immunohistochemical analysis of L5 spinal cord revealed an attenuation in both astrocytic (Fig. 6) and microglial activation (Fig. 7) at 10 days post-transection with both systemic 1 and 10 mg/kg propentofylline treatment compared with saline treatment (Table 1). A similar decrease in both microglial and astrocytic activation at day 10 post-transection was observed with intrathecal 1- and 10-μg propentofylline treatment, but not with intrathecal 0.1-μg propentofylline treatment (Table 2). The doses of propentofylline that attenuated glial activation also inhibited mechanical allodynia in both the systemic and central prevention based models. Immunohistochemical analysis of tissue from the existing pain paradigm revealed a marked attenuation of astrocytic and microglial activation in 1 and 10 mg/kg propentofylline-treated animals compared with 0.1 mg/kg propentofylline or vehicle treated animals (Table 3) at day 20 post-transection. Analysis of spinal microglial activation with densitometry illustrated that 1 and 10 mg/kg propentofylline attenuated both ipsilateral and contralateral microglial activation and trended toward significance (Fig. 8).

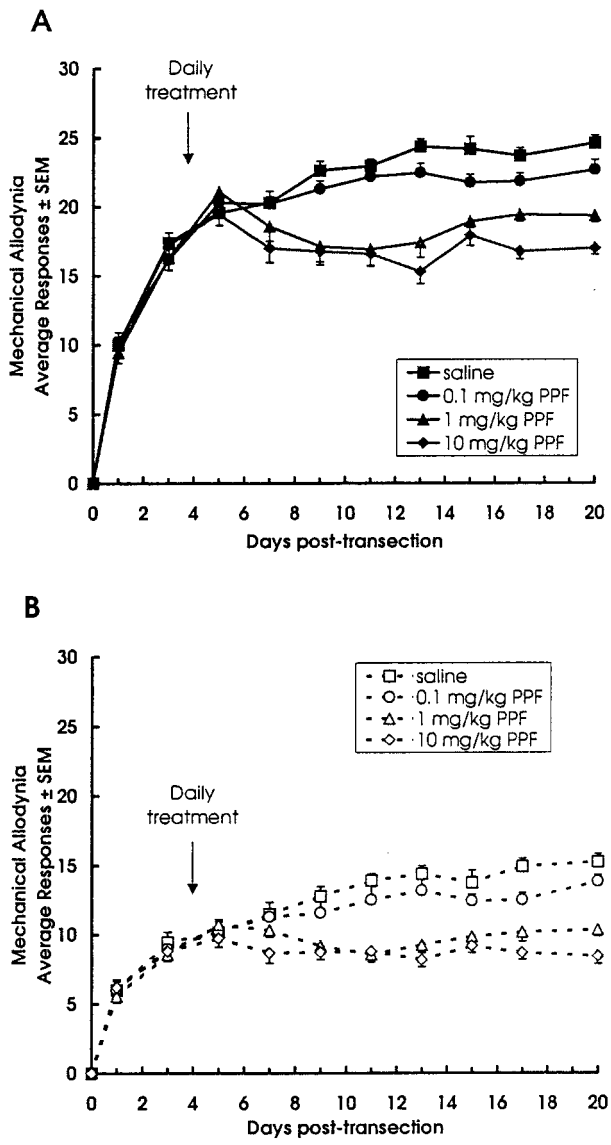

Fig. 4. Systemic treatment of existing allodynia. Male Holtzman rats received daily i.p. 0.1, 1, or 10 mg/kg propentofylline or saline ($n = 12$/treatment) initiated on day 4 post-L5 spinal nerve transection. Treatment with both 1 and 10 mg/kg propentofylline resulted in an overall statistically significant ($p < 0.01$) decrease in both 12- (A) and 2-g (B) von Frey filament-induced mechanical allodynia compared with saline or 0.1 mg/kg propentofylline treatment. Mechanical allodynia is reported as the average number of paw withdrawals out of 30 ± S.E.M. Day 0 mechanical allodynia represents baseline pretransection responses.

Discussion

This study examined the potential therapeutic value of propentofylline in the treatment of neuropathic pain using an L5 spinal nerve transection rodent model of neuropathic pain. Propentofylline was effective in reducing allodynia in preventative and existing pain paradigms following peripheral nerve transection. Propentofylline administered centrally, by lumbar puncture, was more effective in attenuating allodynia than systemic application. This observation supports a central mechanism for propentofylline in this model of neuropathic pain. In addition, the antiallodynic properties of propentofylline at 15 h post-administration support a mechanism of action that includes modulation of intracellu-

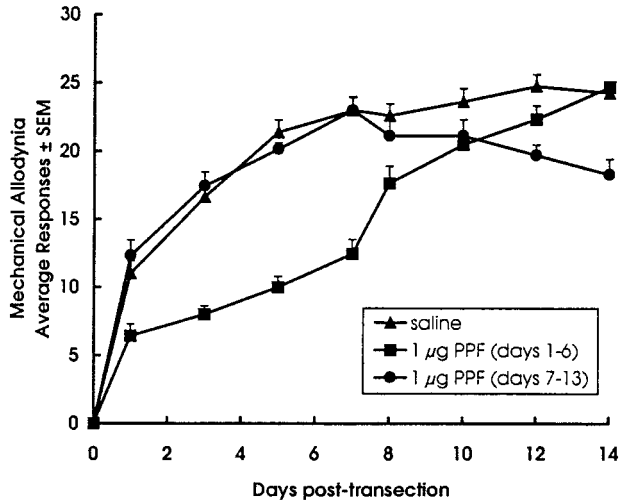

Fig. 5. In a 7-day crossover study 10 μg of propentofylline administered intrathecally in a preventative paradigm (daily intrathecal injection initiated 1 day prior to surgery and continued to day 6) produced a statistically significant ($p < 0.01$, $n = 8$/treatment) reduction in mechanical allodynia compared with saline-treated controls. At day 7 post-transection, treatment was initiated in the crossover animals and continued until day 14. These animals display a significant ($p < 0.01$) reduction in mechanical allodynia at day 12 and 14 post-transection compared with vehicle-treated animals. Mechanical allodynia is reported as the average number of paw withdrawals out of 30 ± S.E.M. Day 0 mechanical allodynia represents baseline pretransection responses.

lar second messenger signaling since the half-life of propentofylline and its active metabolite is about 1 h.

Similar to work completed with propentofylline in ischemia models (DeLeo et al., 1987; Wu et al., 1999), in this present study, propentofylline was found to inhibit both microglial and astrocytic activation in the L5 spinal cord at 10 and 20 days post-L5 spinal nerve transection. Inhibition of glial activation correlated with doses of propentofylline that were antiallodynic. These data suggest that propentofylline attenuated allodynia through an inhibition of central glial activation. Inhibition of glial activation by propentofylline has been attributed to propentofylline's action as a selective phosphodiesterase (PDE) inhibitor. It exerts a powerful inhibition of the cAMP-splitting PDE subtype IV (Meskini et al., 1994) and apparently also of the cGMP-degrading subtypes, which may potentiate intracellular cAMP elevation by a secondary cGMP-mediated blockade of PDE subtype III (Schubert et al., 1997, 2000). Strengthening of cAMP-dependent signaling in cultured microglial cells by the addition of either membrane-permeable dibutyryl-cAMP or propentofylline has been shown to decrease microglial proliferation and activation (Si et al., 1996). Analogously, in astrocyte cultures, application of dibutyryl-cAMP or propentofylline transforms activated phenotypes to differentiated phenotypes (Schubert and Rudolphi, 1998). Activated astrocytes exhibit high proliferation rates, lack of cellular processes, and ion channel patterns lacking K^+ and Cl^- channels. In contrast, dibutyryl-cAMP-treated differentiated astrocytes exhibit well ramified cellular processes and expression of K^+ and Cl^- channels (Ferroni et al., 1995). These K^+ and Cl^- channels are very important for stabilization of the membrane potential, as well as for effective glutamate uptake and ion homeostasis in the neuronal synapse. Excessive synaptic glutamate can contribute to neuronal hyperactivity and sensitization that are important elements in the maintenance of persistent pain states.

In addition, propentofylline may directly regulate proinflammatory cytokine synthesis and release through its action as a phosphodiesterase inhibitor and augmentation of cAMP signaling. This is supported by work in which application of either propentofylline or dibutyryl-cAMP has been found to inhibit lipopolysaccharide-induced release of tumor necrosis factor (TNF), interleukin (IL)-1β, and oxygen radicals in cultured microglia (Schubert and Rudolphi, 1998; Schubert et al., 2000). In addition, increased cAMP-dependent signaling has been shown to augment the expression of anti-inflammatory IL-10 (Platzer et al., 1999). In a peripheral nerve injury model, application of IL-10 at the site of injury was found to decrease TNF at the sight of injury in a pattern that paralleled decreased hyperalgesia (Wagner et al., 1998). Thus, it is possible that propentofylline augments anti-inflammatory cytokine production that in turn further down-regulates proinflammatory cytokine production.

The production of proinflammatory cytokines TNF and IL-1β, which causes the deleterious escalation of a pathological glia activation from microglia to astrocytes, is not only thought to aggravate secondary neuronal damage (Schubert et al., 1997, 2000) but also is important in the generation of central sensitization and pain behaviors (DeLeo and Colburn, 1996; Colburn et al., 1997; DeLeo et al., 1997). IL-1β and TNF contribute to central sensitization through both direct actions on neuronal receptors as well as indirectly through their interactions with glial cells. Directly, IL-1β and TNF are capable of inducing Substance P expression and axonal transport (Ding et al., 1995; Jeanjean et al., 1995). Indirectly, TNF and IL-1β can feed back on both microglia and astrocytes to sustain activation as well as to up-regulate inducible nitric-oxide synthase and cyclooxygenase-2 expression (Tonai et al., 1999). This results in increased levels of prostaglandins and nitric oxide, which are potent neuronal sensitizing molecules. In addition, TNF has been found to increase intracellular Ca^{2+} and depolarization in astrocytes, which leads to aberrant astrocytic uptake of glutamate from the synaptic cleft (Fine et al., 1996; Koller et al., 1996). Thus, propentofylline may be antiallodynic in this model of neuropathic pain by inhibiting glial activation and thus, preventing production of downstream nociceptive mediators such as inducible nitric-oxide synthase, cyclooxygenase-2, and proinflammatory IL-1β and TNF, as well as directly inhibiting synthesis and secretion of proinflammatory cytokines.

As an atypical methylxanthine, propentofylline also functions as an adenosine reuptake inhibitor (Parkinson et al., 1993). This is potentially important because adenosine has been proposed to have a role in neuropathic pain. A recent clinical study found decreased levels of adenosine in the cerebrospinal fluid of neuropathic pain patients compared with patients suffering from nervous system lesions without pain and non-neuropathic pain patients (Guieu et al., 1996). In a phase 1 clinical safety trial, intrathecal adenosine was found to decrease the area of secondary allodynia resulting from mustard oil (Rane et al., 1998). Adenosine presynaptically inhibits the release of Substance P and glutamate release while postsynaptically decreasing the action of Substance P and glutamate (Sawynok, 1998). Inhibition of nociceptive Substance P and glutamate release and action can potentially attenuate central sensitization and the resultant development of pain.

In rodent studies, both A_1 and A_{2a} adenosine receptor

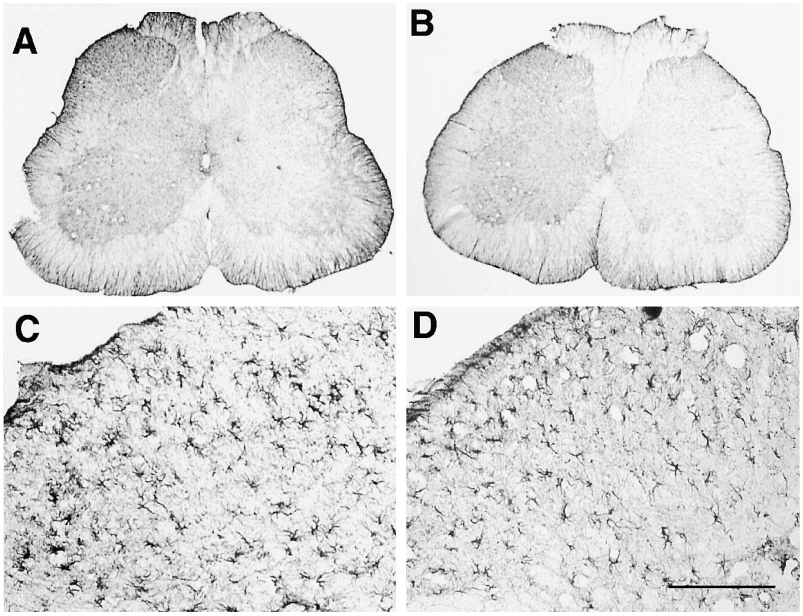

Fig. 6. Attenuation of astrocytic activation by propentofylline. Propentofylline treatment in either an existing or preventative, systemic or central administration paradigm resulted in mild-to-moderate ipsilateral astrocytic activation (B and D) compared with the intense ipsilateral astrocytic activation in the L5 spinal cord of saline-treated (A and C) male rats at 10 or 20 days post-transection. Scale bar, 150 μm (C and D) and 1000 μm (A and B).

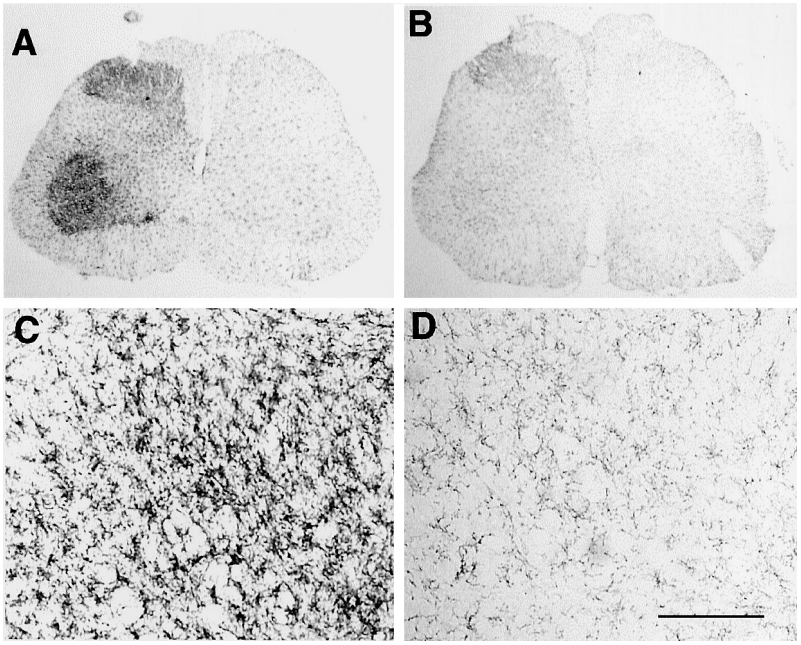

Fig. 7. Attenuation of microglial activation by propentofylline. Propentofylline treatment in either an existing or preventative, systemic or central administration paradigm resulted in mild-to-moderate ipsilateral microglial activation (B and D) compared with the intense ipsilateral microglial activation in the L5 spinal cord of saline-treated (A and C) male rats at 10 or 20 days post-transection. Scale bar, 100 μm (C and D) and 1000 μm (A and B).

agonists have been found to be antiallodynic in both inflammatory and neuropathic pain models (Sawynok, 1998). In addition, A_{2a} receptor agonists have been shown to be anti-inflammatory (Sawynok, 1998). Signaling through the A_{2a} receptor also strengthens cAMP-dependent signaling by activation of adenylate cyclase. However, in a gerbil model of ischemia, inhibition of glial activation was found to be independent of adenosine receptor activation since the nonspecific adenosine receptor antagonist theophylline did not reverse the actions of propentofylline (DeLeo et al., 1988). However, this does not preclude a role for adenosine and adenosine receptor activation in augmenting inhibition of proinflammatory cytokine production via propentofylline's action as a phosphodiesterase inhibitor.

In addition to inhibiting glial activation and proinflammatory cytokine production in the CNS, propentofylline has been shown to reduce leukocyte recruitment in an adenosine A_{2a} receptor-dependent manner in a zymosan model of peritonitis (Zhang et al., 1996). This is likely through cAMP-mediated down-regulation of proinflammatory cytokines. IL-1β and TNF induce the expression of chemotactic factors and intercellular adhesion molecule (ICAM), which are vital for leukocyte capture and extravasation to the site of injury (Aschner, 1998; Fassbender et al., 1999).

A similar mechanism in which proinflammatory cytokines induce a cascade resulting in leukocyte extravasation is observed in the injured CNS. TNF and IL-1β have been found to regulate ICAM expression, on both activated microglia and activated astrocytes, when the CNS is exposed to both ischemic (Fassbender et al., 1999; Yang et al., 1999) and immunologic challenge (Aschner, 1998; Khoury et al., 1999; Raivich et al., 1999). ICAM expression is an important facilitator for astrocytes to function as antigen-presenting cells in intracerebral immune responses. In addition, IL-1β is also an

TABLE 1

Immunohistochemistry results for microglial activation and astrocytic activation in the L5 spinal cord at day 10 post-transection in animals treated with systemic intraperitoneal propentofylline daily from 1 day prior to transection to day 10 post-transection

Scoring is as follows (see Colburn, 1997, for representative scoring photomicrographs): o represents normal unactivated tissue, + represents mild activation, ++ represents moderate activation, and +++ represents intense activation.

Treatment Animal id	Microglial Activation (OX-42)	Astrocytic Activation (GFAP)
Saline		
A1	++	+++
A5	++	+++
B1	+++	+++
C1	+++	+++
C5	++	++
1 mg/kg propentofylline		
A4	+++	++
A6	+	++
B4	++	+++
B6	+	+++
C4	+++	+++
C6	++	++
10 mg/kg propentofylline		
A3	++	++
B2	+	++
B3	+++	++
C2	+	++
C3	+++	+++

TABLE 2

Immunohistochemistry results for microglial activation and astrocytic activation in the L5 spinal cord at day 10 post-transection in animals treated with central intrathecal propentofylline daily from 1 day prior to transection to day 10 post-transection

Scoring is as follows (see Colburn, 1997, for representative scoring photomicrographs): o represents normal unactivated tissue, + represents mild activation, ++ represents moderate activation, and +++ represents intense activation.

Treatment Animal id	Microglial Activation (OX-42)	Astrocytic Activation (GFAP)
Saline		
I3	+++	+++
I4	+	+++
J3	+++	+++
J4	+++	+++
K3	+++	+++
0.1 μg propentofylline		
Q1	+++	++
Q3	+++	+++
Q6	++	+++
R3	++	+++
R4	++	+++
1 μg propentofylline		
I1	+	++
I6	+	+
J1	++	++
J6	++	+++
K1	+	++
10 μg propentofylline		
Q2	++	+++
Q8	++	+
R2	+	+
R5	++	++
R7	++	++

important activating signal in T cell-dependent antigen-specific immune responses (Aschner, 1998). Further downstream, xanthine derivatives have been found to inhibit the generation of IL-2 and down-regulate the immune response (Iwaz et al., 1986). The exact role for immunocompetence within the CNS in persistent neuropathic pain states is still to be determined. However, propentofylline may function to decrease allodynia through mechanisms that inhibit neuroimmune interactions.

TABLE 3

Immunohistochemistry results for microglial activation and astrocytic activation in the L5 spinal cord at day 20 post-transection in animals treated with systemic intraperitoneal propentofylline from day 4 to day 20 post-transection

Scoring is as follows (see Colburn, 1997, for representative scoring photomicrographs): o represents normal unactivated tissue, + represents mild activation, ++ represents moderate activation, and +++ represents intense activation.

Treatment Animal id	Microglial Activation (OX-42)	Astrocytic Activation (GFAP)
Saline		
A10	+++	++
C2	++	+++
C6	+++	++
C10	+++	++
C14	++	++
A14	++	+++
0.1 mg/kg propentofylline		
A13	+	+
C1	+++	+++
B9	+	++
C5	+	+
C9	+++	++
B5	+++	+++
1 mg/kg propentofylline		
C16	++	++
B8	++	++
C4	+	+
C8	+	+
C12	++	++
10 mg/kg propentofylline		
A11	+	++
D3	++	o
C15	+	+++
C7	+	+
C11	++	++
A15	+++	o
B7	+	+

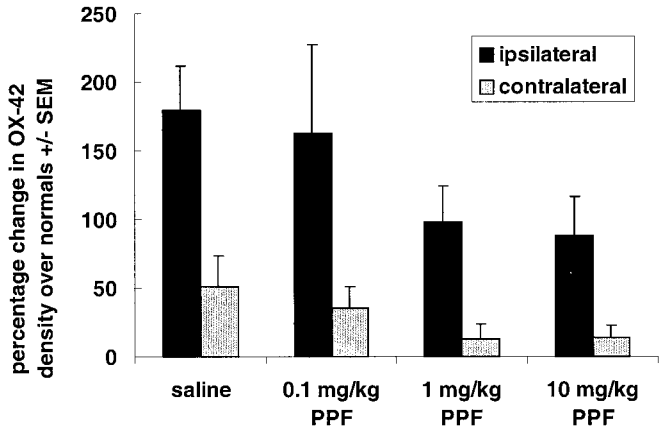

Fig. 8. Percentage of change in microglial activation, as determined using densitometry and OX-42 as the marker of activation, compared with normal naïve animals. Daily intraperitoneal administration of 1 and 10 mg/kg propentofylline, from day 4 to day 20 post-transection, reduced both ipsilateral and contralateral microglial activation compared with vehicle-treated or 0.1 mg/kg propentofylline-treated animals. All tissue compared with densitometry were stained in a single immunohistochemical run to ensure that results represent a change in staining density in injured tissue and not variations in normal staining.

The literature provides at least three potential mechanisms by which propentofylline can strengthen cAMP-dependent signaling and decrease central sensitization. First, propentofylline can inhibit glial activation, which can potentially reduce synthesis and release of oxygen radicals and prostaglandins as well as maintain astrocytic buffering capabilities. Second, propentofylline can inhibit proinflammatory TNF

and IL-1β synthesis and secretion while increasing anti-inflammatory IL-10 expression either via adenosine-independent or adenosine-dependent mechanisms. Third, propentofylline can potentially decrease neuroimmune interactions through an adenosine-dependent pathway. The multitude of potential effects of propentofylline makes the search for mechanisms of antiallodynia complex but highlights the importance of developing novel therapeutic agents for neuropathic pain that modulate both glial activation and proinflammatory cytokine expression.

In summary, this study has shown that propentofylline is a novel antiallodynic agent that is capable of modulating glial activation in the spinal cord following peripheral nerve transection. It is most likely that glial modulation is due to propentofylline-induced strengthening of cAMP signaling. Future studies will assess the effects of propentofylline on the downstream effectors of glial activation such as proinflammatory and anti-inflammatory cytokine expression as well as other neuroimmune interactions. In contrast to opioid treatment, propentofylline does not produce adverse effects such as sedation, tolerance, or physical dependence (Park and Rudolphi, 1994). In addition, in this rodent model of neuropathic pain, once daily administration of propentofylline decreased mechanical allodynia at 15 h post-treatment, highlighting a long-lasting antiallodynic effect and a novel mechanism of second messenger modulation in the treatment of neuropathic pain.

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