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Inhibition of glutamate carboxypeptidase II (NAALADase) protects against dynorphin A-induced ischemic spinal cord injury in rats

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Abstract

Glutamate carboxypeptidase (GCP) II (EC 3.4.17.21), which is also known as *N*-acetylated-α-linked acidic dipeptidase (NAALADase), hydrolyses the endogenous acidic dipeptide *N*-acetylaspartylglutamate (NAAG), yielding *N*-acetyl-aspartate and glutamate. Inhibition of this enzyme by 2-(phosphonomethyl) pentanedioic acid (2-PMPA) has been shown to protect against ischemic injury to the brain and hypoxic and metabolic injury to neuronal cells in culture, presumably by increasing and decreasing the extracellular concentrations of NAAG and glutamate, respectively. Since both NAAG and GCP II are found in especially high concentrations in the spinal cord, injuries to the spinal cord involving pathophysiological elevations in extracellular glutamate might be particularly responsive to GCP II inhibition. Lumbar subarachnoid injections of dynorphin A in rats cause ischemic spinal cord injury, elevated extracellular glutamate and a persistent hindlimb paralysis that is mediated through excitatory amino acid receptors. We therefore used this injury model to evaluate the protective effects of 2-PMPA. When coadministered with dynorphin A, 2-PMPA significantly attenuated the dynorphin A-induced elevations in cerebrospinal fluid glutamate levels and by 24 h postinjection caused significant dose-dependent improvements in motor scores that were associated with marked histopathological improvements. These results indicate that 2-PMPA provides effective protection against excitotoxic spinal cord injury.
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1. Introduction

Traumatic or ischemic injury to the central nervous system (CNS) greatly increases extracellular concentrations of the excitatory amino acid neurotransmitter glutamate which, through excessive stimulation of excitatory amino acid receptors, evokes a cascade of disrupted intracellular processes promoting cell dysfunction and death (reviewed by McIntosh et al., 1996, 1998). Although a number of pharmacological agents that selectively antagonize the action of glutamate at its receptors have been shown to reduce injury in experimental models of cerebral ischemia and trauma, none have yet been effective in clinical trials due primarily to unacceptably serious side effects (Olney et al., 1989). Consequently, to some extent, the focus for development of therapeutic agents has shifted away from *N*-methyl-D-aspartate (NMDA) receptor antagonists and has been expanded to include alternative means to interrupt this glutamate-mediated excitotoxic cascade.

One promising alternative strategy is inhibition of the extracellular metalloproteinase glutamate carboxypeptidase (GCP II; EC 3.4.17.21), which is also known as *N*-acetylated-

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α-linked acidic dipeptidase (NAALADase). GCP II readily hydrolyses the endogenous acidic dipeptide *N*-acetylaspartylglutamate (NAAG), which is abundantly distributed throughout the brain and spinal cord, yielding *N*-acetylaspartate and glutamate (Robinson et al., 1987). In addition to serving as a potentially important source of synaptic glutamate under pathophysiological conditions, NAAG is also a neuropeptide that fulfills most of the criteria for classification as a neurotransmitter (reviewed by Coyle, 1997; Neale et al., 2000). Notably, it is localized to glutamatergic neuronal pathways and is released in a calcium-dependent manner upon synaptic stimulation. NAAG is a mixed agonist/antagonist at NMDA receptors (Sekiguchi et al., 1989; Burlina et al., 1994; Puttfarcken et al., 1993; Valivullah et al., 1994; Koenig et al., 1994) and also is an agonist at mGluR3 group II metabotropic glutamate receptors (Wroblewska et al., 1993, 1997; Bruno et al., 1998a), which have been shown to mediate neuroprotection through presynaptic inhibition of glutamate release and through elicitation of transforming growth factor-β (TGF-β) synthesis and release (Bruno et al., 1997, 1998a,b). Thus, inhibition of GCP II provides a promising therapeutic strategy through which glutamate-mediated excitotoxicity can be countered by several complementary means. This premise has been supported by studies from several laboratories in which the selective potent CGP II inhibitor, 2-(phosphonomethyl) pentanedioic acid (2-PMPA), has been shown to protect against a variety of neuronal insults involving excitotoxicity in vitro and in vivo (Slusher et al., 1999; Tortella et al., 2000; Thomas et al., 2001a,b). Notably, 2-PMPA has been shown to protect against neuronal injury caused by middle cerebral artery occlusion in vivo (Slusher et al., 1999) and by metabolic inhibition, hypoxia or excitatory amino acid exposure in vitro (Tortella et al., 2000; Thomas et al., 2001a,b).

Dynorphin A has been implicated as a mediator in the pathophysiology of spinal cord injury, based in part on its ability to cause persistent flaccid hindlimb paralysis and extensive neuronal injury after injection into the spinal subarachnoid space of rats (Faden and Jacobs, 1984; Herman and Goldstein, 1985; Long et al., 1988; Stevens and Yaksh, 1986). The onset of dynorphin A-induced hindlimb paralysis coincides with a significant degree of ischemia, anaerobic metabolism and vascular deterioration (Long et al., 1987, 1994; Thornhill et al., 1989), indicating that these neurological deficits and neuropathological changes are primarily attributable to dynorphin A-induced reductions in blood flow with resultant ischemic neuronal injury. Moreover, coadministration of the potent vasodilator hydralazine has been shown to substantially eliminate the anaerobic metabolic, paralytic and neuropathological responses to dynorphin A (Long et al., 1994). Collectively, these findings reveal that dynorphin A is among several peptides that, as a result of direct vasospastic actions on the microvasculature of the rat central nervous system, provide a convenient pharmacological means to induce and study graded, localized ischemic spinal cord injury (Long et al., 1992).

Activation of the NMDA glutamate receptor appears to play a pivotal role in the pathophysiology associated with dynorphin A-induced spinal cord injury. Concentrations of the excitatory amino acid neurotransmitters glutamate and aspartate are significantly increased shortly after the onset of dynorphin A-induced hindlimb paralysis (Long et al., 1994). In addition, a variety of competitive and noncompetitive inhibitors of the NMDA receptor complex have been shown to significantly improve recovery of hindlimb motor function following dynorphin A injection into the spinal subarachnoid space (Bakshi and Faden, 1990a,b; Caudle and Isaac, 1988; Long et al., 1994; Long and Skolnick, 1994). These findings indicate that dynorphin A-induced rat spinal cord injury appears to be a reproducible injury model that is potentially well-suited for the study of NMDA receptor pharmacology and excitotoxic phenomena. Therefore, we utilized this injury model in these experiments to assess the therapeutic utility of GCP II inhibition as a means to counter excitoxic injury in the rat spinal cord.

2. Materials and methods

2.1. Spinal subarachnoid injections and neurological assessments

Male Sprague–Dawley rats (250–300 g) were anesthetized with halothane and dorsal midline incisions were made immediately rostral to the pelvic girdle. Spinal subarachnoid injections were delivered using 30-gauge needles inserted between the lumbar L4–L5 vertebrae. Using the vertebral processes as guides, the needle was advanced to pass into the subarachnoid space surrounding the cauda equina. Correct needle placement was verified by cerebrospinal fluid (CSF) flow from the needle following its insertion. Injections were delivered in a total volume of 22 μl [containing the dynorphin A (20 nmol), 2-PMPA (1–4 μmol) or vehicle and the cannula flush] using a Hamilton microsyringe. Following injections, incisions were treated with the topical antibacterial furazolidone and closed with wound clips. Rapid recovery from the halothane anesthesia enabled neurological evaluations to be made within 5 min of injections.

Neurological function was independently evaluated by blinded observers using a 5-point ordinal scale, with scores being assigned as follows: 4=normal motor function; 3=mild paraparesis, with the ability to support weight and walk with impairment; 2=paraparesis, with the ability to making walking movements without supporting weight; 1=severe paraparesis, in which rats could make limited hindlimb movement, but not walking movement; 0=flaccid paralysis, with complete absence of any hindlimb movement. Neurological scores were recorded 2 h and 24 h following dynorphin A injection.

2.2. CSF collection and amino acid measurements

For amino acid measurements, CSF was removed from anesthetized rats through a 30 gauge needle reinserted through the L4–L5 vertebral space. CSF (approximately 70 μl) was collected on ice for approximately 10 min beginning 15 min after spinal subarachnoid injections of dynorphin A. CSF was immediately acidified to 0.2 N with addition of 2N HCl and frozen until assayed. An aliquot was mixed with L-α-aminoadipate, as an internal standard, and amino acids were measured by pre-column derivatization with *o*-phthaldialdehyde using an automated injector as previously described (Robinson et al., 1993). After separation by reverse phase chromatography using a stepped gradient, amino acids were detected by a McPherson Model FL-750BX fluorescence detector. Peak areas were integrated using Millenium software (Waters/Millipore, Milford, MA, U.S.A.). External standards were injected after every fifth specimen and a standard curve was constructed to calculate the amounts of internal standard and amino acids in each specimen.

2.3. Neuroanatomical assessments

For histopathological assessments, rats were anesthetized with ketamine and xylazine (70 and 6 mg/kg, i.m., respectively) 72 h after injection of dynorphin A, then perfused transcardially with physiological saline followed immediately by a 10% formalin solution for fixation. The lumbosacral cord was removed, and a block of tissue including the lumbar enlargement, corresponding to L5–L6, was mounted for cross sectional analysis. Cresyl violet-stained sections (6 μm) were prepared from five sites at 800 μm intervals within this 5 mm block. In sections prepared from sites 2 and 4, neurons were counted within 0.25 mm^2 square areas within the left and right ventrolateral portion of the anterior horn gray area by a blinded observer. Neurons with a cell dimension >25 μm and a visible nucleus were counted. A mean cell number was determined for each rat using the left and right counts from the two sample sites.

2.4. Chemicals

Dynorphin A (1–17) and somatostatin were purchased from Peninsula Laboratories (Belmont, CA) and 2-PMPA was provided by Guilford Pharmaceuticals (Baltimore, MD).

2.5. Data analysis

Differences in the nonparametric neurological scores among treatment groups were determined by means of the Mann–Whitney *U*-test and the Kruskal–Wallis test. Ambulatory frequency data were assessed using the chi-square test. Neuronal cell counts and concentrations of amino acids in CSF samples were compared using analysis of variance, and post hoc comparisons were made using the least significant differences test.

3. Results

At the 20-nmol dose used throughout these experiments, dynorphin A routinely caused flaccid hindlimb paralysis persisting beyond 24 h (Fig. 1) and significant neuronal injury throughout the lumbosacral cord (Fig. 4 and Table 1). When coadministered with dynorphin A, 2-PMPA caused significant dose-dependent improvements in motor scores by 2 and 24 h postinjection (Fig. 1). Flaccid paralysis was still acutely evident following 2-PMPA cotreatment; the 2-PMPA-induced improvement in neurological recovery became increasingly apparent between 2 and 24 h post-injection. Rats showing improvements in hindlimb motor function at 2 h showed equal or greater improvement at 24 h (i.e., improvement was not transient). Although not shown in Fig. 1, motor scores recorded at 24 h postinjection generally remained unchanged through the next 48 h. Parenthetically, higher doses of 2-PMPA alone (>4 μmol) also caused neurological deficits. In these cases, the neurological deficits caused by PMPA were indistinguishable from those caused by dynorphin A; hindlimb and tail flaccidity were evident within 10 min of injection and persisted through 24 h postinjection. When on the basis of their assigned neurological scores (0–4) rats were categorized as ambulatory (3 or 4) or nonambulatory (0, 1 or 2), at 24 h postinjection 80% of the rats cotreated with 4 μmol of 2-PMPA could walk in contrast to 17% of the rats cotreated with saline vehicle (Fig. 2).

To rule out the possibility that protection against dynorphin A-induced spinal cord injury might result from interactions of 2-PMPA and dynorphin A independent of the

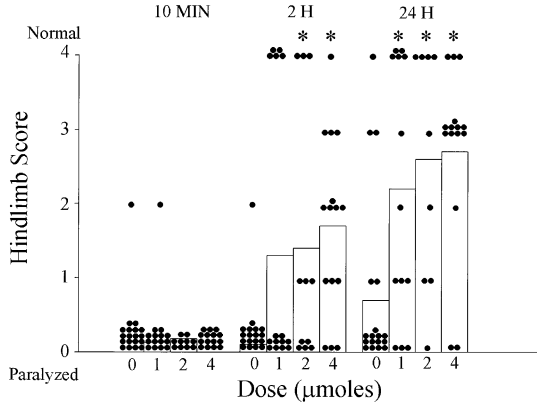

Fig. 1. Protective effects of 2-PMPA following spinal subarachnoid injection of 20 nmol of dynorphin A. In the doses indicated, 2-PMPA was coadministered as a spinal subarachnoid injection with the dynorphin A. Neurological scores assigned to individual rats are depicted as points, and mean scores for dose groups are represented by bar heights. Kruskal–Wallis comparison of hindlimb scores revealed that all three doses of 2-PMPA significantly improved hindlimb motor scores by 24 h postinjection relative to rats cotreated instead with saline vehicle. *$P<0.05$.

Table 1
Effects of PMPA on dynorphin A-induced loss of neuronal cell bodies in the L5–L6 anterior horn

Treatment group	*N*	Neuron count
Veh/Veh	6	7.2±0.6
Dyn/Veh	6	2.2±0.9[a]
Dyn/2-PMPA (4 μmol)	11	6.8±0.7[b]
Dyn/2-PMPA (2 μmol)	6	8.1±1.1[b]
Dyn/2-PMPA (1 μmol)	7	4.8±1.0

Neuronal cell bodies exceeding 25 μm in any one dimension were counted within 0.25 mm^2 areas within the left and right ventrolateral portion of the anterior horn gray area by a blinded observer. Cell numbers were averaged for the left and right counts from two sample sites. Coinjection of PMPA (2 and 4 μmol) significantly protected against the loss of neuronal cell bodies recorded in rats injected with dynorphin A (20 mol) in combination with saline vehicle.

[a] $P<0.01$ when compared to Veh/Veh-injected rats.

[b] $P<0.01$ when compared to Dyn/Veh-injected rats.

peptide-induced ischemia, we also evaluated the effects of 2-PMPA in rats receiving spinal subarachnoid injections of somatostatin, which is a structurally unrelated peptide that has also been shown to cause hindlimb paralysis, vasospasm and ischemic spinal cord injury in rats (Long, 1988; Long et al., 1992). As shown in Fig. 3, 2-PMPA also significantly improved recovery of hindlimb motor function in rats paralyzed after spinal subarachnoid injection of 25 nmol of somatostatin, which like dynorphin A causes flaccid hindlimb paralysis in association with spinal cord ischemia (Long et al., 1992).

The protective effects of 2-PMPA were also clearly evident by histopathological assessment of spinal cords removed from rats 72 h after dynorphin A injection. In contrast to the necrosis, hemorrhage and cellular infiltration typically seen in cords of dynorphin A-injured rats cotreated with saline vehicle, significant sparing of neurons in both dorsal and ventral horns were observed in rats cotreated with 4 μmol of 2-PMPA (Fig. 4). The spongy rarefaction of white matter that was characteristically seen in dynorphin A-injured cords was also less evident in the cords of rats

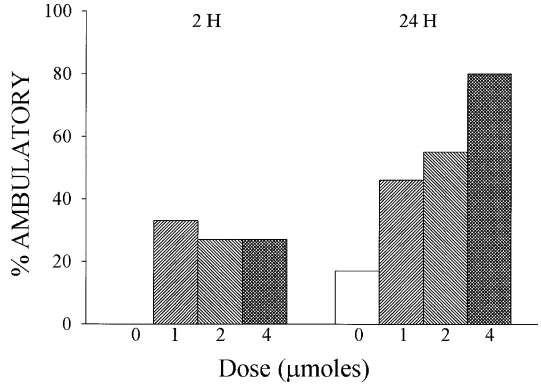

Fig. 2. On the basis of the hindlimb scores depicted in Fig. 1, rats were classified as ambulatory (3 or 4) or nonambulatory (0, 1 or 2). Recovery of hindlimb motor function was clearly evident at 2 and 24 h postinjection. Chi-square analysis revealed a significant difference in ambulatory rates among treatment groups at 24 h after dynorphin A injection ($P<0.005$).

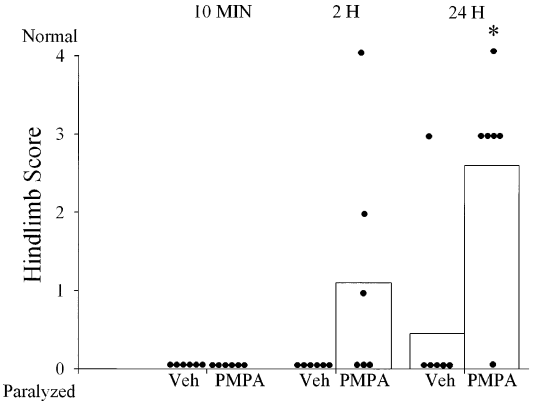

Fig. 3. Protective effects of 2-PMPA following spinal subarachnoid injection of 25 nmol of somatostatin. Either 2-PMPA (4 μmol) or saline vehicle (Veh) was coadministered as a spinal subarachnoid injection with the somatostatin. As in Fig. 1, neurological scores assigned to individual rats are depicted as points, and mean scores for dose groups are represented by bar heights. Mann–Whitney *U*-test comparison of hindlimb scores revealed that 2-PMPA significantly improved hindlimb motor scores by 24 h postinjection relative to rats cotreated instead with saline vehicle. *$P<0.05$.

cotreated with 2-PMPA. Cell counts in the ventrolateral portions of the L5–L6 anterior horn quantitatively revealed the deleterious and salutary effects of dynorphin A and 2-

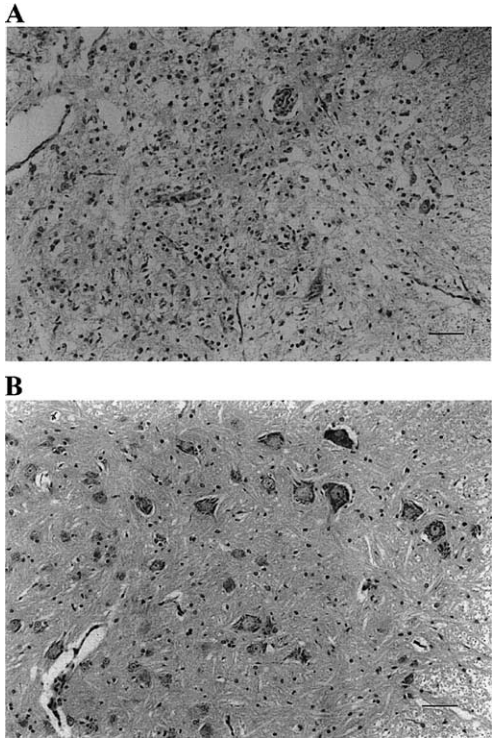

Fig. 4. Photomicrographs of the fifth lumbar spinal segment from rats exposed to dynorphin A in combination with either saline vehicle (**A**) or 4 μmol of 2-PMPA (**B**). At 72 h postinjection, dynorphin A-treated rats characteristically exhibited extensive necrosis of the gray matter and spongy rarefaction of the white matter. In contrast, dynorphin A-injected rats cotreated with 4 μM 2-PMPA typically displayed overall protection of cells in the lumbar spinal cord; viable motor neurons and relatively limited edema in the surrounding white matter were readily apparent. Calibration bar: 50 um (in lower right corner).

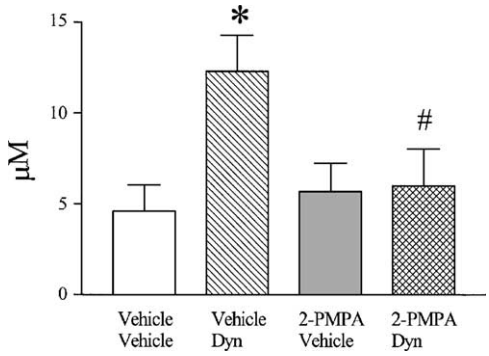

Fig. 5. Effects of 2-PMPA on dynorphin A-induced elevations in spinal cord CSF glutamate concentrations. Spinal cord CSF was removed 15–20 min following injection of saline vehicle (Veh) or 2 μmol of 2-PMPA in combination with either saline vehicle (Veh) or 20 nmol of dynorphin A (Dyn). N=4–7 subjects per treatment group. *P<0.01 when compared to saline-injected, nonparalyzed rats. $^{\#}P$<0.05 when compared to rats injected with dynorphin A in combination with saline vehicle.

PMPA, respectively (Table 1). Rats paralyzed by dynorphin A had a significant loss of large neuronal cell bodies in this region, whereas the dynorphin A-injected rats cotreated with PMPA had a dose-related preservation of these cells, that in all likelihood constitute α-motoneurons that are directly involved in locomotion.

Relative to the CSF samples removed from vehicle-injected control rats, following dynorphin A injection the extracellular concentrations of glutamate in spinal cord CSF significantly increased by approximately three-fold, to 12.3 μM (Fig. 5 and Table 2). Since dynorphin A does not contain glutamate as one of its constituent amino acids, this increase in glutamate cannot be attributed to simple metabolism of the injected peptide. Dynorphin A also caused significant increases in concentrations of tyrosine, arginine and asparagine, which, in contrast to glutamate, are contained within the dynorphin A peptide structure and would be predictably elevated following metabolism of injected dynorphin A by aminopeptidases or basic endopeptidases (Gillan et al., 1985; Chesneau et al., 1994; Berman et al., 1999). When coinjected with dynorphin A, 2-PMPA (which by itself did not alter CSF concentrations of any of the amino acids) significantly reduced the dynorphin A-induced elevations in glutamate. In contrast, PMPA did not reduce the dynorphin A-induced increases in either arginine, tyrosine or asparagine.

4. Discussion

As has been previously shown with ischemic injuries to the brain (Slusher et al., 1999; Thomas et al., 2001a) and with hypoxic or metabolic injuries to neurons in culture (Slusher et al., 1999; Tortella et al., 2000; Thomas et al., 2001b), 2-PMPA exhibited dose-dependent neuroprotective actions against the spinal cord injuries and persistent neurological deficits caused by spinal subarachnoid injection of dynorphin A. 2-PMPA did not prevent the immediate induction of hindlimb and tail flaccidity by dynorphin A. Rather, as was previously observed with several NMDA and glycine receptor antagonists (Bakshi and Faden, 1990a,b; Isaac et al., 1990; Long et al., 1994; Long and Skolnick, 1994), significant motor recovery was first clearly evident about 2 h after dynorphin A injection, suggesting that the protective effects of this compound resulted from the interruption of secondary pathophysiological events rather than from a direct interruption of or interference with the initial pharmacological actions of dynorphin A that trigger an ischemic insult to the lumbosacral cord.

Similar to earlier findings with NMDA and glycine receptor antagonists (Long et al., 1994; Long and Skolnick, 1994), the 2-PMPA-induced improvements in neurological function were appreciable; 24 h following administration of 4 μmol of 2-PMPA 80% of rats were ambulatory, in contrast to a 17% recovery recorded in the vehicle-treated animals. These findings reinforce other indications of the importance of excitotoxicity within the sequence pathophysiological

Table 2
2-PMPA effects on CSF amino acid concentrations (mM) during dynorphin A-induced spinal cord ischemia

Amino acid	Veh/Veh	Veh/Dyn	PMPA/Veh	PMPA/Dyn
Asp	6.0±1.2	7.4±1.9	3.4±1.1	6.4±1.9
Glu	4.5±1.3	12.3±2.0[a]	5.7±1.6	6.0±2.1[b]
Asn	22.4±4.0	49.5±7.5[a]	38.5±1.6	35.3±8.9
Ser	130.3±21.7	149.9±21.5	162.5±14.5	131.8±23.9
Gln	3170.6±389.0	3236.7±164.8	2890.6±163.9	3032.2±435.0
Tyr	10.6±3.5	51.5±5.7[a]	26.9±4.2	39.9±11.2[a]
Arg	174.0±39.8	369.7±40.5[a]	243.4±34.7	332.5±54.8[a]
Ala/Tau	221.0±112.7	892.7±236.5	383.8±210.9	666.8±169.4
GABA	16.5±3.6	13.4±2.7	10.3±2.7	10.0±3.1
Trp	6.75±5.0	9.7±2.2	11.2±3.8	4.9±1.5
Phe	10.2±3.1	19.8±2.0	18.0±2.1	26.3±6.9
Ile	20.6±5.4	28.5±10.1	21.3±5.4	33.1±9.6
Leu	16.9±7.5	44.8±7.0	23.8±8.5	28.6±9.4

[a] P<0.01, significantly different when compared to Veh/Veh.
[b] P<0.05, significantly different when compared to Veh/Dyn.

events yielding persistent hindlimb paralysis in this model of spinal cord injury. Histopathological evaluation of spinal cords removed from 2-PMPA-cotreated rats similarly and consistently revealed a significant sparing of neurons in both dorsal and ventral horns relative to that seen in the dynorphin A injured rats cotreated with vehicle (Fig. 4 and Table 1).

In addition to its deleterious effects on the vasculature that lead to ischemic injury, dynorphin A has also recently been shown to interact directly with the NMDA receptor complex (Chen and Huang, 1998; Tang et al., 1999; Shukla et al., 1997). To rule out the possibility that the salutary effects of 2-PMPA in the present study might have resulted from any potential influence of 2-PMPA on responses to direct interactions of dynorphin A with the NMDA receptor complex (independent of the peptide-induced ischemia), we also evaluated the effects of 2-PMPA in rats receiving spinal subarachnoid injections of somatostatin, which is a structurally unrelated peptide that has also been shown to cause hindlimb paralysis, vasospasm and ischemic spinal cord injury in rats (Long, 1988; Long et al., 1992). Recovery of hindlimb motor function was similarly significantly improved in somatostatin-treated rats coinjected with 2-PMPA, confirming the efficacy of this agent and indicating that the therapeutic effects seen were not likely to have derived from any direct interactions of 2-PMPA and dynorphin A with one another or with the NMDA receptor complex.

The differences in CSF glutamate concentrations across treatment groups are consistent with the neuroprotective effects of 2-PMPA arising from inhibition of GCP II. Specifically, 2-PMPA eliminated the dynorphin A-induced increases in glutamate in CSF samples taken from the spinal cord, as would be expected if the hydrolysis of NAAG serves as a primary source of the elevated extracellular glutamate seen under these pathophysiological conditions. 2-PMPA did not cause a change in CSF glutamate concentrations in rats not injected with dynorphin A, indicating that the effects of the drug on extracellular glutamate are restricted to the disrupted milieu associated with injury. Obviously, from these data alone we cannot rule out the possibility that the diminished elevations in glutamate concentrations might have also resulted from alternative actions of 2-PMPA that served to lessen the severity of the dynorphin A-induced injury and in turn reduce the associated release and extracellular accumulation of glutamate. In addition, as reviewed by Obrenovitch and Urenjak (1997), the importance of rapid transient increases in extracellular glutamate to excitotoxic mechanisms is not universally agreed upon. Nevertheless, it is important to note that, although not measured in the present study, in previous related work with rat middle cerebral artery occlusion, 2-PMPA-induced reductions in extracellular glutamate occurred concomitantly with elevations in extracellular NAAG concentrations (Slusher et al., 1999), as would be predicted with GCP II as the site of drug action.

In addition to the therapeutic benefits derived from reductions in extracellular glutamate, the neuroprotection resulting from GCP II inhibition can also arise from the concomitantly accumulated NAAG acting at several different excitatory amino acid receptors. NAAG has been shown to function as a partial agonist/antagonist at NMDA receptors (Sekiguchi et al., 1989; Burlina et al., 1994; Puttfarcken et al., 1993; Valivullah et al., 1994; Koenig et al., 1994) and to additionally act as an agonist at group II metabotropic glutamate receptors, with greatest selectivity being seen for group II mGluR3 receptors, which are found on both neuronal and glial cells (Wroblewska et al., 1993, 1997, 1998). As a partial agonist (with reduced efficacy), NAAG would act as an NMDA receptor antagonist under conditions where synaptic concentrations of glutamate are at or near saturation. Group II metabotropic glutamate receptors have been proposed to elicit neuroprotective actions through inactivation of voltage-sensitive calcium channels (Schoepp and Conn, 1993), inhibition of cAMP formation (Buisson and Choi, 1995) and presynaptic reduction of glutamate release (Sanchez-Prieto et al., 1996). In addition, the importance of glial mGluR3 receptors to the neuroprotective effect of NAAG and 2-PMPA in certain experimental situations has recently been recognized and has been shown to involve the synthesis and release of TGF-β (Bruno et al., 1998b; Thomas et al., 2001a,b). Collectively, this expanding body of data points to NAAG potentially and substantially contributing to the neuroprotective effects of 2-PMPA in a multidimensional manner.

The relative distribution of NAAG and NAALADase in the central nervous system, wherein highest concentrations are found in the spinal cord (Miyake et al., 1981; Koller and Coyle, 1984, 1985), suggest that a neuroprotective intervention targeting these endogenous mediators might be particularly well-suited as a therapeutic means of suppressing glutamate-mediated excitotoxicity associated with spinal cord injuries and other neuropathological conditions. Consistent with these expectations, we observed the selective GCP II inhibitor 2-PMPA to significantly reduce elevated extracellular glutamate and to greatly improve recovery from dynorphin A-induced ischemic spinal cord injury. These data reinforce and extend earlier indications pointing to the potential importance of NAAG and GCP II under pathophysiological circumstances associated with excitotoxic actions of extracellular glutamate. As previously proposed (Slusher et al., 1999), a broad spectrum "upstream" approach to counter glutamate excitotoxicity through GCP II inhibition offers several advantages over receptor-targeted therapies, and if well-tolerated, provides an attractive new strategy for drug development.

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was conducted under a protocol approved by an Institutional Animal Care and Use Committee, in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals. All procedures were carried out in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals, NRC Publication, 1996 edition. The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense (para. 4-3), AR 360-5.

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